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NITROGEN FIXATION BY AZOTOBACTER AS INFLUENCED BY MOLYBDENUM AND VANADIUM[1]

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INTRODUCTION

The stimulating action of molybdenum on nitrogen fixation by *Azotobacter*, first reported by Bortels (*7*),[2] has since been observed by a number of other investigators. In addition, the beneficial effect of this element has been demonstrated by Bortels (*12*), for nitrogen fixation by *Nostoc;* by Steinberg (*36*, *37*) and Steinberg and Bowling (*39*), for the growth of *Aspergillus niger;* by Bortels (*10*), Obraztzova and others (*32*), Bobko and Savvina (*6*), and Bertrand (*4*), for legumes; and by De Rose, Eisenmenger, and Ritchie (*19*), Arnon (*1*), Arnon and Stout (*2*), and Piper (*33*), for other higher plants. Van Niel (*40*), Bortels (*9*), and Konishi and Tsuge (*27*, *28*) have found that the addition of molybdenum or vanadium to soils may increase bacterial numbers and nitrogen fixation. Less is known about the effect of vanadium than of molybdenum on organisms; but whenever direct comparisons have been made on *Azotobacter* or legumes, vanadium has had a smaller effect than molybdenum on nitrogen fixation (*8*, *9*, *10*, *13*, *15*).

Previous investigations by Burk (*13*) and Burk and Horner (*15*, *16*) of the influence of molybdenum and vanadium on *Azotobacter* consisted chiefly of growth-rate experiments with the Warburg manometric technique. Those studies showed that in a nitrogen-free medium growth and nitrogen fixation were increased markedly by molybdenum or vanadium but that these catalytic elements did not appreciably affect growth when the medium contained fixed nitrogen. A very low concentration of either molybdenum or vanadium was effective.

Other investigators who have reported studies of the effect of molybdenum or vanadium on *Azotobacter* have used the usual Erlenmeyer flask culture technique almost exclusively. The outstanding studies of this type are those of Bortels (*8*, *11*), who determined the effect of varying concentrations and combinations of molybdenum, vanadium, iron, and other elements on nitrogen fixation or utilization and on pigment production, and those of Krzemieniewski and Kovats (*30*) and Kovats (*29*), who investigated the relative effects of iron, molybdenum, vanadium, humic acids, and the ash of the humates on nitrogen fixation and on efficiency of sugar utilization.

Experiments conducted with the Warburg respiration technique and with the stagnant long-time culture method are not necessarily directly comparable. The purpose of the studies reported herein was (1) to

[1] Received for publication October 13, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 191.

attempt to confirm some of the results obtained by others, particularly those of the investigators mentioned in the preceding paragraph, by using their methods, and (2) to obtain information on other phases of the problem not yet studied or inadequately studied. These latter include the influence of molybdenum in relation to age of culture, its impurity in the media, and strain variation. The effective concentration range of both molybdenum and vanadium for several typical strains is given. These data are needed for a proper evaluation of previous work and for a better understanding of the nutritional or catalytic role of these elements. The question whether molybdenum and vanadium are specific catalysts for the nitrogen-fixation process or whether they also favor the growth of *Azotobacter* supplied with combined nitrogen will not be considered here. Aside from workers in this laboratory (*13*, *14*, *15*, *16*), only Birch-Hirschfeld (*5*) and Bortels (*8*) have considered this phase of the problem. This subject is now under active investigation. In all the work reported below, the organisms were grown on nitrogen-free media, where growth is dependent upon nitrogen fixation; hence *the two terms "growth" and "nitrogen fixation" are used interchangeably.*

MATERIALS AND METHODS

The two culture media chiefly employed are designated as A and B. Medium A was prepared as follows: Eight-tenths of a gram of dipotassium phosphate (K_2HPO_4), 0.2 gm. of monopotassium phosphate (KH_2PO_4), 0.2 gm. of magnesium sulfate ($MgSO_4.7H_2O$), and 0.1 gm. of calcium sulfate ($CaSO_4.2H_2O$) were added to 1 liter of distilled water and thoroughly shaken. After the undissolved material had settled, the clear solution was decanted off as desired. It contained about 15 percent less phosphate and 40 percent less calcium than had been added. Just before use, an additional 20 percent of distilled water, 10 or 20 p. p. m. of synthetic humate (containing 10 percent of iron) (*25*), 0.05 percent of neutralized lactic acid,[3] and 1 or 2 percent of sucrose or glucose were added to the clear solution. The constituents of this medium remain soluble so that daily observations of bacterial growth or turbidimetric analyses can be made. Medium B was similar to that of Bortels and was prepared by adding 1.0 gm. of dipotassium phosphate (K_2HPO_4), 0.5 gm. of magnesium sulfate ($MgSO_4.7H_2O$), 1.0 gm. of calcium carbonate ($CaCO_3$), 0.02 gm. of iron sulfate ($FeSO_4.7H_2O$), and 10 or 20 gm. of glucose or sucrose to 1 liter of distilled water. Medium B was always turbid because of the excess of calcium carbonate. When molybdenum was added to either medium it was supplied as sodium molybdate ($Na_2MoO_4.2H_2O$); vanadium was added as the ortho or meta vanadate. Modifications of media A and B that were used in some experiments are described in connection with the individual experiments.

Erlenmeyer flasks of 125- or 250-cc. capacity, containing 15 or 25 cc. of media, respectively, served as culture vessels. This gave a layer of liquid 3 to 6 mm. in depth. The flasks were plugged with cotton and sterilized in an autoclave at 10 to 15 pounds' pressure for 15 to 30 minutes. When glucose was present, the lower pressure and shorter time were used to prevent excessive decomposition.

[3] A small amount of sodium lactate has been found to be advantageous in maintaining a slightly alkaline reaction for some strains of *Azotobacter* that tend to produce acidity. The pH of the cultures varied between 6.6 and 8.6; in general, cultures with the most growth had the highest pH values.

The two strains of *Azotobacter* employed in the majority of the experiments were *A. chroococcum* Beij. B–8 [4] and *A. vinelandii* Lip. V–1.[5] Other strains, used for comparison, were *A. chroococcum* B–5, B–7, and B–10; [4] *A. vinelandii* B–4, B–6, and B–9; [4] *A. agile* Beij. K–1 (*26*); [6] *A. agilis atypica* Kluyver and Van den Bout K–2; [6] *A. chroococcum* C–4 and C–5; [7] and *A. chroococcum* C–12 and C–15.[8]

Inoculation of the media in the experimental flasks was accomplished by adding one or two drops of a 1- or 2-day liquid culture that had been growing for some time on medium A with frequent transfers. The cultures were incubated at 28° to 31° C. for various periods, depending upon the purpose of the experiments. The purity of the cultures, especially of the inoculum, was checked by frequent inoculation into a peptone-meat extract medium as well as by microscopic examination.

Analyses were made on aliquots of the cultures at the end of the incubation period after sufficient distilled water had been added to restore them to their original volume. The dry matter produced was obtained by centrifuging, washing once with distilled water, and drying in an electric oven at 80°–100° C. The unoxidized carbohydrate in the centrifugate was determined by means of the Von Fellenberg (*20*) chromic acid oxidation procedure. This method, though not strictly specific for the determination of sugar, is convenient and gives a close approximation in the case of *Azotobacter*, which is known to oxidize glucose and sucrose or their intermediate oxidation products almost completely to CO_2 and H_2O (*31*). Centrifugates from cultures that have obtained maximum growth usually give a reaction, upon chromic acid oxidation, equivalent to up to 5 percent of the initially added sugar. This is probably non-sugar and largely represents carbon originating from the excreted nitrogenous compounds (*24*). The oxidizable carbon was calculated as glucose on the basis of 1 cc. of 0.1 N potassium dichromate ($K_2Cr_2O_7$) as equivalent to 0.75 mg. of glucose. All nitrogen, dry-matter, and carbohydrate analyses were based on 100 cc. in order to facilitate comparisons. Turbidimetric measurements were made by means of a Bausch and Lomb nephelometer. The standard for comparison was prepared by shaking an excess of pulverized bentonite in distilled water, allowing it to stand overnight, and using the decanted suspension or suitable dilutions of it. Humate was added, comparable to that in the culture medium, to provide approximately the same tint. The most turbid standard was arbitrarily called 800 units, which is equivalent to the turbidity given by a culture of *Azotobacter* containing about 14 to 18 mg. of nitrogen or 100 to 150 mg. of dried bacteria per 100 cc. These turbidity units are not necessarily comparable with those reported in previous publications from this laboratory. Colorimetric pH determinations were made with various La Motte indicators and standards. Total nitrogen was determined by the micro-Kjeldahl method.

[4] Obtained from O. P. Maximova, of the laboratory of A. N. Bach, U. S. S. R. Academy of Sciences, Moscow.

[5] Original strain isolated by J. G. Lipman and obtained from him about 18 years ago.

[6] Received from H. W. Reuszer, formerly of the Colorado Agricultural Experiment Station, who had obtained them from A. J. Kluyver, of Delft, Netherlands.

[7] Obtained from the National Collection of Type Cultures, Lister Institute, London, England, as Nos. 4183 and 1865.

[8] Obtained from N. R. Smith, of the Division of Soil Microbiology, Bureau of Plant Industry, U. S. Department of Agriculture, as Nos. 12 and 15.

EXPERIMENTAL RESULTS

AGE OF CULTURE

Figures 1 and 2 show the effect of molybdenum upon nitrogen fixation by *Azotobacter chroococcum* B–8 and *A. vinelandii* V–1 in relation to age of culture. The data presented in these figures are from numerous experiments carried out over a period of 3 years

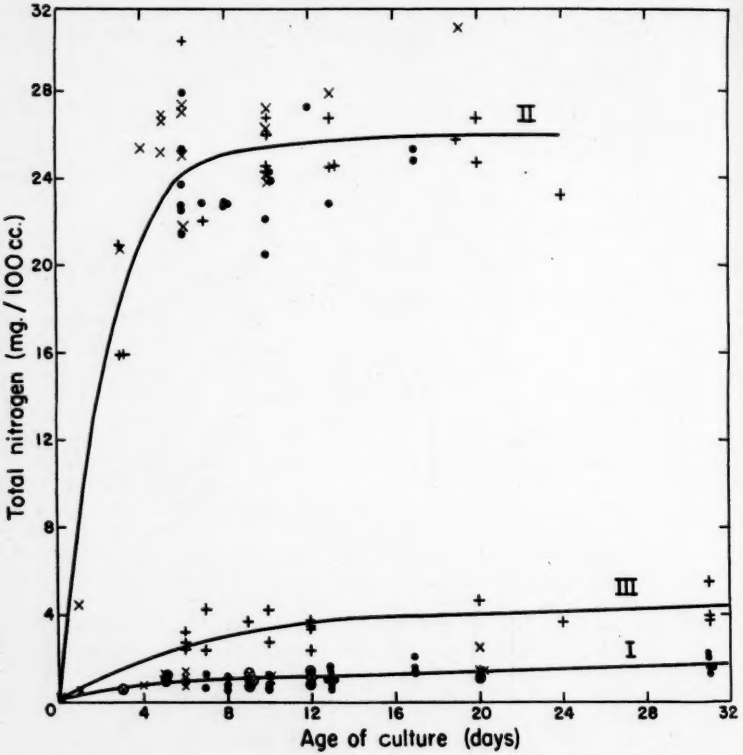

FIGURE 1.—Total nitrogen fixed by *Azotobacter chroococcum* B–8 as a function of age, with and without added molybdenum: Curve I, no added molybdenum, and curve II, 1 or 4 p. p. m. of molybdenum, various samples of sucrose or glucose serving as sources of energy; curve III, no added molybdenum, sucrose 1 serving as the source of energy. Symbols: • = 2 percent glucose; ⊙ = 1 percent glucose; + = 2 percent sucrose 1; × = 2 percent other sucroses; ⊗ = 1 percent other sucroses.

Although the conditions in the various experiments were similar, both media A and B with modifications were employed, and several samples of chemically pure sucrose or glucose designated in the legends were used. The usual experimental variations further tended to give a scattering of points around the curves.

The preponderant influence of molybdenum over any of these other variables is evident from the extreme divergence (a maximum of

thirtyfold to fortyfold) between curves I and II. Curve III of figure 1 indicates that one sample of sugar, sucrose 1, which consistently caused 50 to 100 percent or more growth than any of the other energy sources in the absence of added molybdenum, probably contained an appreciably greater impurity. Further evidence for this will be given, below. Both strains of *Azotobacter* responded similarly to molybdenum

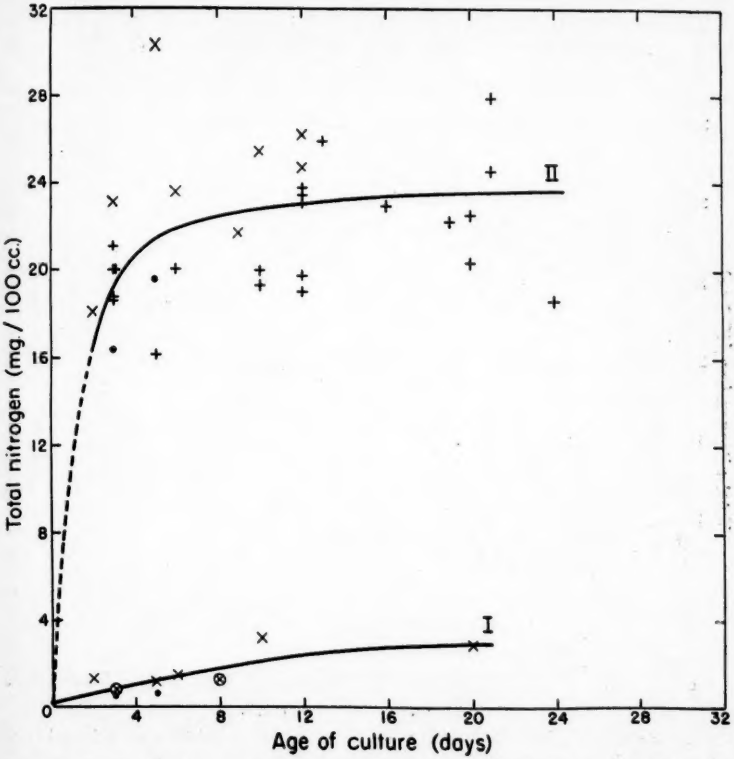

FIGURE 2.— Total nitrogen fixed by *Azotobacter vinelandii* V–1 as a function of age, with and without added molybdenum: Curve I, no added molybdenum, and curve II, 1 or 4 p. p. m. of molybdenum, various samples of sucrose or glucose serving as the sources of energy. Symbols: • = 2 percent glucose; + = 2 percent sucrose 1; × = 2 percent other sucroses; ⊗ = 1 percent other sucroses.

attaining maximum growth by about the sixth to eighth day. In the absence of added molybdenum, analyses have shown 1 to 1. 5 gm. out of the original 2 gm. per 100 cc. of carbohydrate still remaining after 31 days. Since the energy supply is not limiting in the latter case, cultures with either 1 or 2 percent fall equally well on curve I. The cultures without molybdenum continue to grow slowly for weeks after those with adequate molybdenum have consumed their energy supply and ceased growing, but the greatest increment in nitrogen

fixation takes place during the first few days. This would seem to indicate that if no molybdenum impurity were present there would be no growth. *A. vinelandii* V–1, as a rule, gives a little more nitrogen fixation with no added molybdenum and a slightly lower maximum when it is optimum than is observed for *A. chroococcum* B–8; the latter organism also usually grows somewhat more rapidly during the first 1 or 2 days, at least as evidenced by turbidity observable.

The relation of age of culture to total nitrogen fixed by another strain, *Azotobacter vinelandii* B–6, in the presence and absence of

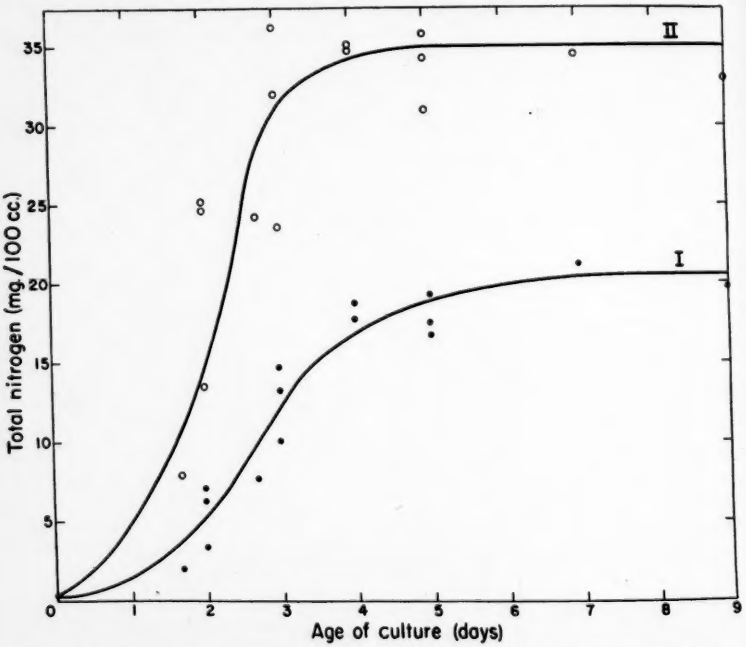

FIGURE 3.—Total nitrogen fixed by *Azotobacter Vinelandii* B–6 as a function of age, with and without added molybdenum; energy source, 2 percent sucrose 2: Curve I, no added molybdenum; curve II, 1 p. p. m. molybdenum.

molybdenum, is shown in figure 3. The maximum nitrogen fixation with 2 percent of sugar and adequate molybdenum was only a little greater for this strain than for *A. vinelandii* V–1 (fig. 2), but in the absence of molybdenum the relatively large amount of growth of the former in contrast to the latter is very striking. In figure 3 it will be observed that on the second or third day the fixation in the absence of added molybdenum was 30 to 40 percent of that with molybdenum; on the fifth to sixth day it was 50 percent or more. Such high nitrogen fixation in the presence of mere traces of molybdenum as impurity, amounting to 20 to 22 mg. of nitrogen per 100 cc., in contrast to 1 to 2 mg. of nitrogen fixed by the other two organisms, shows a very different molybdenum requirement for this organism.

MODIFICATION AND PURIFICATION OF MEDIA

Several attempts were made to lower the molybdenum impurity in the media and to obtain more convincing proof of its essentiality for nitrogen fixation by *Azotobacter*. In the writers' earlier work, 0.2 mg. of sodium chloride (NaCl) per liter had been added to medium A, but recent tests have shown not only that it is unnecessary but that it may contain a trace of molybdenum. It was also observed that freshly prepared medium A usually gave somewhat less growth in the absence of molybdenum than if this medium were prepared from inorganic solutions that had stood for some time in contact with the undissolved sediment. The use of water that had been redistilled

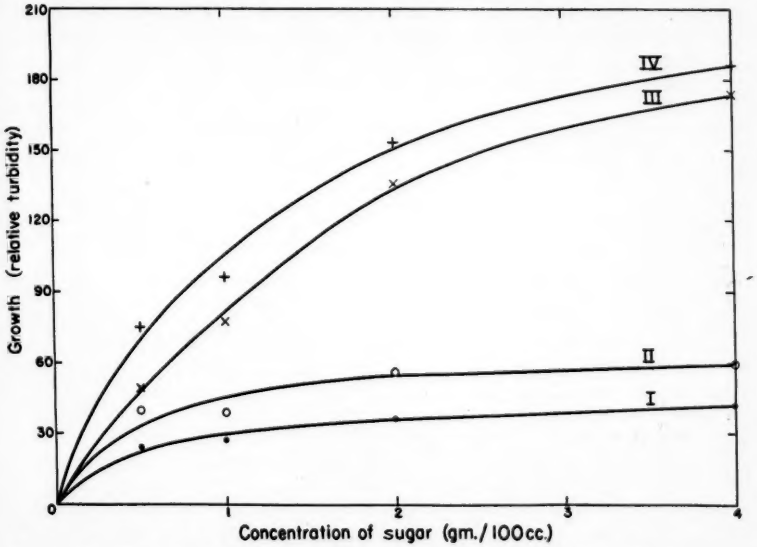

FIGURE 4.—Growth of *Azotobacter chroococcum* B–8 as a function of sugar concentration in the absence of added molybdenum: Curve I, sucrose 2, 2-day culture; curve II, sucrose 2, 5-day culture; curve III, sucrose 1, 2-day culture; curve IV, sucrose 1, 5-day culture.

from pyrex vessels gave the same results as ordinary distilled water. The sources of calcium or iron were reversed for the two media in some experiments, and other sources were tested, but there was no consistent effect either on the maximum nitrogen fixation by *Azotobacter* obtainable with optimum molybdenum or on the minimum without molybdenum.

The sugar, which is present in many times the concentration of any other constituent of the media, may well furnish considerable molybdenum impurity, as is indicated in figure 1. Out of about a dozen different c. p. sugars tested, including sucrose, glucose, mannite, and levulose, all except sucrose 1 gave about the same small growth on molybdenum-deficient media. Unfortunately, this particular sugar had been in use for some time before it was compared with other samples. Figure 4 shows the striking difference in the response of

Azotobacter chroococcum B–8 to increasing concentrations of sucrose 1 and sucrose 2. The latter caused less than a twofold increase in growth as the concentration was raised from 0.5 to 4 percent, whereas the former gave a threefold effect. The relatively small change between the 2- and 5-day cultures indicates little tendency for the cultures with sucrose 2 to approach with age those with sucrose 1, at least not while the energy supply is adequate. In a similar series of cultures, where optimum molybdenum was added and heavy growth and a rapid utilization of sugar occurred, there was no essential difference between the growths with the two sugars. It would seem, therefore, that the improved development of molybdenum-deficient cultures produced by sucrose 1 is due to a greater molybdenum impurity. A concentration of sugar greater than 4 percent tends to inhibit growth of *A. chroococcum* B–8, with or without molybdenum, and hence the shape of the curves in figure 4 may be influenced to some extent by an approach to this inhibiting range.

TABLE 1.—*Nitrogen fixation by 10-day cultures of Azotobacter chroococcum B–8 as affected by charcoal purification of sugar and salts of the medium*

Sucrose No.	Sugar (2 percent) purified	Inorganic salts purified	Total nitrogen (mg. per 100 cc.) with—		Sucrose No.	Sugar (2 percent) purified	Inorganic salts purified	Total nitrogen (mg. per 100 cc.) with—	
			No Mo	1 p. p. m. Mo				No Mo	1 p. p. m. Mo
3	−	−	1.29	26.0	3	+	+	1.02	.....
3	+	−	.99	26.2	3	+	+[1]	1.00	23.8
3	−	+[1,2]	1.32	.....	3	+[3]	+[1]	.93	.....
3	−	+	1.36	.....	1	−	+[1]	4.23	24.5
3	−	+[1]	1.34	24.0	1	+	+[1]	1.03	24.1
3	+	+[1,2]	.93	.....	1	+[4]	+[1]	1.17	.....

[1] Calcium was supplied as $CaCl_2$ instead of $CaSO_4 \cdot 2H_2O$.
[2] Salt solutions collected after filtering through the charcoal 2 to 3 hours; others collected during first 2 to 3 hours.
[3] Not treated with charcoal but recrystallized from water with alcohol.
[4] Purified with Pfanstiehl charcoal; others purified with Norit.

Preliminary attempts to remove this apparent molybdenum impurity from the media by means of Steinberg's (*35*) procedure of calcium carbonate ($CaCO_3$) precipitation or by letting *Azotobacter* remove the impurity and then re-using the media were unsuccessful. However, if sugar solutions were allowed to percolate through charcoal, and the sugar was then recrystallized from alcohol, the growth of *Azotobacter* in the media containing the purified sugars and no added molybdenum was definitely lowered. When optimum molybdenum was added to the media the charcoal treatment of the sugars caused no significant effect on the growth of the organisms. Table 1 summarizes the results of an experiment with *Azotobacter chroococcum* B–8 in which various combinations of purified and unpurified salts and sugars were employed as media. The charcoal treatment of the salts had practically no effect, but the purified sucrose 1 gave markedly less growth in the absence of molybdenum than did the unpurified sample. Even with sucrose 3, slightly less growth in molybdenum-deficient media was obtained when this sugar was treated with charcoal, although recrystallization from alcohol alone was nearly as efficient. It is interesting that the nitrogen fixed by the organisms in the absence of molybdenum was nearly the same in the presence

of either of the purified sucroses 1 or 3, and only slightly less than with unpurified sucrose 2. Evidently it is the molybdenum impurity in the various samples of sucrose that is chiefly responsible for their different behavior in supporting nitrogen fixation by *Azotobacter*.

STRAIN VARIATION

The results of two experiments in which 14 strains, comprising 3 species of *Azotobacter*, were compared are shown in table 2. Data are given for total nitrogen fixed, dry matter, cell nitrogen, carbohydrate consumed, and milligrams of nitrogen fixed per gram of sugar utilized for cultures with and without added molybdenum. The two experiments are not strictly comparable because of the different incubation periods, concentrations, and samples of sucrose used. The lower concentration of sugar used in experiment 1 reduced the total molybdenum added as impurity but of course also limited the total nitrogen fixation in the presence of an adequate supply of this element. Bearing in mind these considerations, certain generalizations may be made. It will be noted that when no molybdenum was added the two strains of *Azotobacter agile* and all the strains of *A. vinelandii* except *A. vinelandii* V–1 gave relatively high nitrogen fixation. It would appear that even with sucrose 2, although only one of these strains in each of the two species was tested with the purer sugar, the total nitrogen fixation amounted to 60 to 70 percent of that obtained with optimum molybdenum. On the other hand, the strains of *A. chroococcum*, with sucrose 2, showed a nitrogen fixation in the absence of added molybdenum of about 10 percent or less of that in the presence of an adequate supply of this element. Thus, in this respect *A. vinelandii* B–6 appears to be a typical representative of the *A. agile* and *A. vinelandii* strains studied, and *A. chroococcum* B–8 behaves like the other strains of *A. chroococcum*.

Over a period of years *Azotobacter vinelandii* V–1, as mentioned in the discussions of figures 1 and 2, has usually responded to molybdenum more nearly like the *A. chroococcum* strains than like the other *A. vinelandii* organisms tested. In several recent experiments, however, cultures of this particular strain fixed 10 to 14 mg. of nitrogen per 100 cc. in the absence of added molybdenum. Normal behavior of cultures of *A. chroococcum* B–8, which were tested simultaneously, showed that the changed behavior of *A. vinelandii* V–1 was not due to a higher molybdenum impurity in the medium. Further work will be necessary to determine whether or not *A. vinelandii* V–1 has undergone a permanent variation with respect to the quantitative effect of molybdenum.

The strains of *Azotobacter chroococcum* whose behavior in respect to molybdenum was similar showed certain differences among themselves in other physiological characteristics. For example, strains B–10, C–4, C–12, and C–15 grew especially slowly under the conditions employed, even when supplied with molybdenum. This is evident from table 2, experiment 1, which shows that by the third day all the cultures except these had attained practically maximum growth. *A. chroococcum* C–15 appears to be unique in possessing a lower ultimate nitrogen-fixing ability. Whereas all of the other *A. chroococcum* strains in experiment 1 gave a fixation of 13 to 16 mg. of nitrogen per 100 cc. of medium by the eighth day, when growth had practically stopped, this organism yielded only 9.3 mg. of nitrogen per

100 cc., with carbohydrate still remaining and growth essentially at maximum. Table 2 also shows that the efficiency of carbohydrate utilization was normal. Perhaps this organism requires additional trace elements as reported for several strains of *Azotobacter* by Schröder (*34*).

TABLE 2.—*Comparative effect of molybdenum (1 p. p. m.) on different strains of Azotobacter*

[Figures are for 100 cc. of culture]

Experiment No. and *Azotobacter* species and strain	Age of culture	Total nitrogen fixed with—		Dry weight of cells with—		Nitrogen content of cells with—		Sugar consumed with—		Nitrogen fixed per gram of sugar consumed with—	
		No Mo	Mo	No Mo	Mo	No Mo	Mo	No Mo	Mo	No Mo	Mo
	Days	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Pct.*	*Pct.*	*Gm.*	*Gm.*	*Mg.*	*Mg.*
Experiment 1:[1]											
A. agile K–1	3	7.58	10.7	86	101	7	9	1.07	1.06	7	10
A. vinelandii B–6	3	8.15	13.8	54	85	10	12	1.06	1.06	8	13
A. vinelandii V–1	3	.77	8.2	[2] 7	58	[3] 9	12	.....	1.07	.....	8
A. chroococcum B–5	3	.54	13.1	[2] 3	93	[3] 14	10	.....	.80	.....	16
A. chroococcum B–7	3	.60	14.6	[2] 4	84	[3] 12	12	.....	1.02	.....	14
A. chroococcum B–8	3	.49	15.0	[2] 4	90	[3] 10	13	.....	1.04	.....	14
A. chroococcum B–10	3	.49	10.0	[2] 9	159	[3] 4	4	.....	.65	.....	15
A. chroococcum C–4	3	.37	5.4	[2] 4	43	[3] 8	10	.....	.32	.....	17
A. chroococcum C–5	3	.46	12.7	[2] 4	122	[3] 9	8	.....	.86	.....	15
A. chroococcum C–15	3	.49	6.2	[2] 7	49	[3] 6	11	.....	.39	.....	16
A. agile K–1	8	7.08	10.9	.....	.....	.....	.....	.....	.....	.....	.....
A. vinelandii V–1	8	1.26	10.0	11	59	8	12	.45	1.07	3	9
A. chroococcum B–5	8	.94	13.7	17	75	5	12	.14	1.04	7	13
A. chroococcum B–7	8	.77	.....	[2] 13	.....	[3] 5	.....	.22	.....	4	.....
A. chroococcum B–8	8	1.00	14.7	14	86	6	12	.19	1.06	5	14
A. chroococcum B–10	8	.97	14.3	[2] 19	.....	[3] 4	.....	.38	.....	3	.....
A. chroococcum C–4	8	.77	15.5	[2] 13	108	[3] 5	12	.11	1.05	7	15
A. chroococcum C–5	8	.77	14.7	[2] 14	.....	[3] 4	.....	.14	.....	6	.....
A. chroococcum C–12	8	.80	13.0	[2] 7	.....	[3] 9	.....	.....	.....	.....	.....
A. chroococcum C–15	8	.80	9.3	21	68	3	10	.13	.66	6	14
Experiment 2:[4]											
A. agile K–1	6	10.0	26.0	110	174	9	13	1.00	1.82	10	14
A. agilis atypica K–2	6	7.2	14.3	.....	.....	.....	.....	.....	.....	.....	.....
A. vinelandii B–4	6	9.3	25.0	83	184	10	11	.80	1.70	12	15
A. vinelandii B–6	6	12.3	28.9	.....	.....	.....	.....	.....	.....	.....	.....
A. vinelandii B–9	6	9.3	19.6	.....	.....	.....	.....	.....	.....	.....	.....
A. vinelandii V–1	6	4.1	20.0	.....	.....	.....	.....	.....	.....	.....	.....
A. chroococcum B–8	6	2.7	16.5	.....	.....	.....	.....	.....	.....	.....	.....
A. chroococcum C–4	6	1.9	19.6	22	143	8	14	.26	1.52	7	13
A. chroococcum C–15	6	2.2	9.6	42	87	.....	10	.17	.67	13	14

[1] Initial sugar, 1.10 gm. of sucrose 2 per 100 cc.
[2] Dry matter estimated from turbidity.
[3] Nitrogen content calculated on the basis of cell nitrogen equal to 80 percent of the total nitrogen.
[4] Initial sugar, 2.00 gm. of sucrose 1 per 100 cc.

The dry matter produced by the various strains was usually roughly parallel to the nitrogen fixed, except for *Azotobacter chroococcum* B–10 and C–5. The proportionately greater dry weight of these organisms was due to larger gum formation. To permit satisfactory centrifugation the cultures were acidified to pH 2 to 3, which coagulated the gum with the cells and accounts for the lower nitrogen content of the dry matter. Where molybdenum was present most of the cultures gave a rather uniform nitrogen content of 10 to 12 percent of the dry matter. Many of the molybdenum-deficient cultures were apparently considerably lower in their percentage of nitrogen, but the approximate estimation of both the dry matter and cell nitrogen for the very light cultures reduces the accuracy of the figures.

The efficiency of carbohydrate utilization (milligrams of nitrogen fixed per gram of carbohydrate consumed) varied to some extent for the different cultures but was frequently twofold to threefold greater with molybdenum. Bortels (*8*), Krzemieniewski and Kovats (*30*), and Kovats (*29*) report similar or somewhat greater differences for their organisms with and without molybdenum. This element, therefore, exerts its effect upon both the rate of growth of *Azotobacter* and the total nitrogen fixation, or growth per unit of sugar consumed, the latter very probably being largely a consequence of the former.

EFFECTIVE CONCENTRATION RANGE OF MOLYBDENUM AND VANADIUM

Figure 5 represents graphically the nitrogen fixation of 6-day-old cultures of *Azotobacter chroococcum* B–8 as a function of suboptimal concentrations of molybdenum and vanadium when sucrose 1 and sucrose 2 served as sources of energy. A comparison of the molybdenum curves shows that with the lower range of molybdenum there is considerable divergence between the results with the two sugars, but as the optimum is approached the curves tend to coincide. This is true also for the vanadium curves. If we assume that the nitrogen fixed with no added molybdenum depends upon the impurity in the medium, the apparent trend of all of the curves is to approach zero nitrogen fixation with zero molybdenum or vanadium, and we may extrapolate the curves to their intercept with the horizontal axis and obtain an approximate analysis of this impurity. The difference between the intercepts for the two curves (*b–a*) would indicate the extra impurity supplied by sucrose 1. The estimates thus obtained indicate a maximum of about 0.0002 p. p. m. of molybdenum (or vanadium) contained in the medium with 2 percent of sucrose 2, and 0.0012 p. p. m. with 2 percent of sucrose 1. It is obvious that these are only rough estimates because of the nonlinearity of the curves and culture variabilities, but if the points for curve II are shifted along their horizontal axes for a distance equal to *b–a*, practically all fall on curve I. The same correction might be made with respect to vanadium for curves III and IV, but they do not so nearly coincide. This does not mean, however, that the impurity is solely molybdenum, for curves III and IV may be merely less accurately defined than curves I and II. The estimated 0.001 mg. of molybdenum supplied to the medium by 20 gm. of sucrose 1, while sufficient to yield about 10 percent of the optimum effect, is in reality only a minute trace (0.000005 percent) of impurity. It may be of interest to recall that Steinberg (*38*) reported the presence of 0.00004 percent of molybdenum in the sucrose he used.

The effective suboptimal concentration range for 6-day-old cultures of *Azotobacter chroococcum* B–8 covers the range from 0.0001 p. p. m. (detectable effect) to 1 p. p. m. (essentially optimum). A concentration of about 0.03 p. p. m. yields half-maximum growth, whereas 0.1 p. p. m. gives 80 to 90 percent of the maximum effect (fig. 5, insert). The concentration ranges for molybdenum and vanadium are practically identical, but, as reported previously (*13*), the latter element gives only about two-thirds of the maximum growth produced by molybdenum. The differences between the effects of the two elements at the lower concentrations tend to become somewhat smaller, especially when culturing with less pure sucrose. Bortels (*8*) has

reported very similar concentration ranges for his strains of *A. chroococcum* and *A. vinelandii*, especially at the upper ranges that have been studied most. His optimum concentration of vanadium appears to be somewhat less than that of molybdenum, and he observed toxicity with about 1 p. p. m. of vanadium. In the writers' experiments, no toxicity was observed at several times this concentration. In earlier work in this laboratory, in which the Warburg manometric technique was used for growth-rate studies of *A. vinelandii* V–1, a much lower and narrower effective range of molybdenum was obtained (0.000003 to 0.0001 p. p. m.). More recent experiments

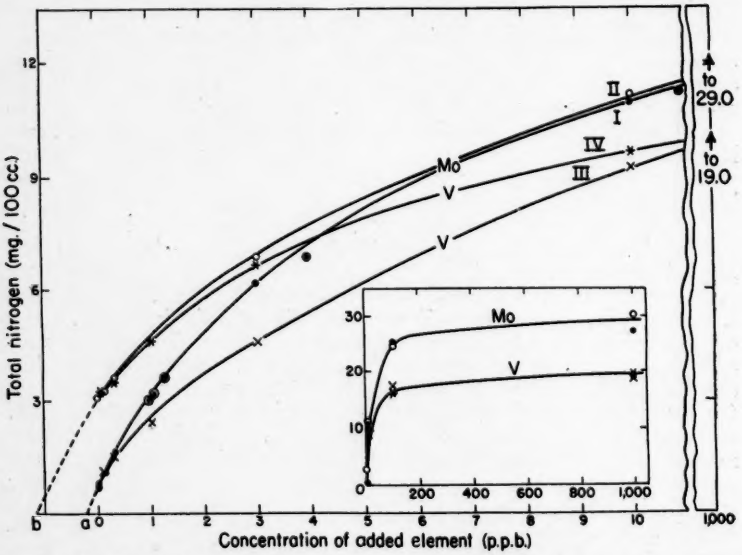

FIGURE 5.—Total nitrogen fixed by 6-day-old cultures of *Azotobacter chroococcum* B–8 as a function of the concentration of molybdenum or vanadium: Curve I, molybdenum with 2 percent sucrose 2; curve II, molybdenum with 2 percent sucrose 1; curve III, vanadium with 2 percent sucrose 2; curve IV, vanadium with 2 percent sucrose 1; ⊙–⊙, a replot of curve II after correction of total nitrogen for molybdenum impurity (*b*–*a*).

with the Warburg technique have indicated some shifting and broadening of this concentration range. *A. vinelandii* V–1 and *A. chroococcum* B–8 respond similarly. As might be expected, a lower concentration of molybdenum is required for the maximum rate of growth of young well-aerated cultures than for the maximum total growth of unaerated long-time Erlenmeyer flask cultures.

Age itself influences the effective suboptimal concentration range of molybdenum, as is shown for *Azotobacter chroococcum* B–8 in figure 6. Although the minimum concentration of molybdenum required to give an effect is about the same for both 1- and 6-day cultures, the optimum concentration for the former is 0.01 or less of that for the latter. The concentration yielding half-maximum growth is likewise shifted from about 0.0013 p. p. m. of molybdenum for the younger cultures to about 0.03 p. p. m. of molybdenum for the older ones.

It has been shown (table 2 and fig. 3) that most strains of *Azotobacter vinelandii* tested were able to fix one-third to two-thirds as much nitrogen in the purest obtainable media as in media with molybdenum added. It might be expected from this that these organisms utilize the molybdenum so much more efficiently that a very low concentration would yield maximum growth, but the tabulation given below shows that this concentration is only slightly if at all lower than the 1 p. p. m. required by *A. chroococcum* B–8. In the light of these data, one might question whether molybdenum should be considered essential for such organisms as *A. vinelandii* B–6. This could be definitely

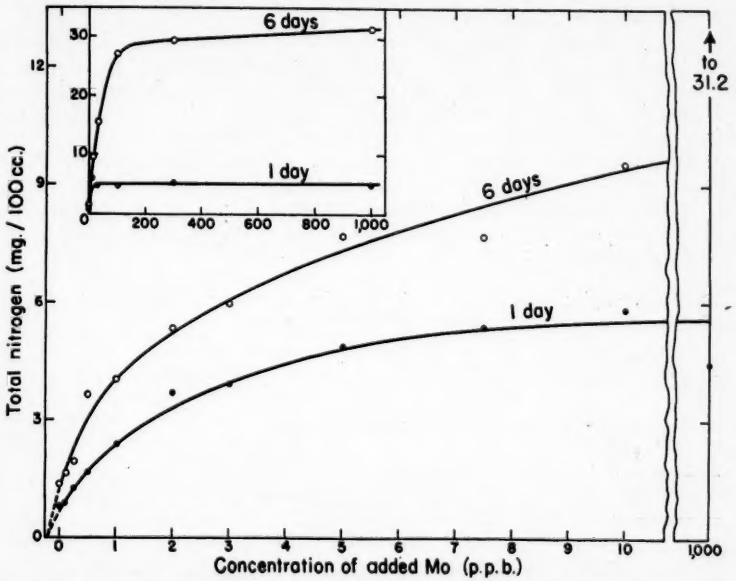

FIGURE 6.— Effect of age upon the effective concentration range of molybdenum for nitrogen fixation by *Azotobacter chroococcum* B–8.

determined, however, only by using media free from all traces of this element.

The effective concentration range of molybdenum for *Azotobacter vinelandii B–6* (5-day cultures) follows:

Mo added (p. p. m.):	*Total N fixed (mg. per 100 cc.)*	Mo added (p. p. m.)	*Total N fixed (mg. per 100 cc.)*
0.0	17. 1	0.01	26. 4
0.00001	18. 4	0.03	29. 2
0.00003	17. 2	0.1	32. 4
0.0001	19. 7	0.3	31. 6
0.0003	20. 5	1.0	32. 6
0.001	21. 2	10.0	33. 1
0.003	24. 5		

RELATION OF IRON TO MOLYBDENUM EFFECT ON NITROGEN FIXATION BY AZOTOBACTER

Burk, Lineweaver, and Horner (*17*, *18*) showed the importance of humic acids as carriers of iron for *Azotobacter*. Later, Horner and

Burk (*23*) worked out the effective concentration range of iron for *Azotobacter vinelandii* (0.02–0.5 p. p. m.) and also reported an apparent effect of molybdenum from traces present as impurities in certain iron salts and humus preparations. This effect had previously been thought to be due entirely to iron stimulation. Since the significance of molybdenum in the nutrition of *Azotobacter* was not fully appreciated at that time, the cultures were as a rule suboptimal with respect to molybdenum. It remained for Bortels (*11*) and Krzemieniewski and Kovats (*30*) to show that both elements must be present in optimal amounts to produce maximum growth of this organism. Some 5 to 10 times the optimal iron concentration previously reported by the writers was found necessary in the presence of adequate molybdenum. Work at this laboratory has confirmed these findings. Table 3 indicates that any suboptimal concentration of molybdenum allows a certain limit for growth beyond which it is not raised by increasing the concentration of iron. Likewise, with suboptimal iron, increasing concentrations of molybdenum are only partly effective until the concentration of iron is raised. There is also some indication from table 3 that the relative suboptimal concentration effects of molybdenum and iron differ to some extent for *A. vinelandii* V–1 and *A. chroococcum* B–8. Bortel's work also indicates such differences between his strains.

TABLE 3.—*Effect of molybdenum in Azotobacter cultures supplied with high and low concentrations of iron*

[Figures are for 100 cc. of culture]

Trace element supplied		Total nitrogen fixed by—			
		A. vinelandii V–1 (5 days) with—		*A. chroococcum* B–8 (13 days) with—	
Mo	Fe [1]	Glucose	Sucrose 1	Glucose	Sucrose 1
P. p. m.	*P. p. m.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0.0002 [2]	0.2	0.51		0.98	
0.0012 [2]	.2		2.80		3.45
4	.2	5.87	3.01	7.67	10.0
0.0002 [2]	4	.65		.85	
0.0012 [2]	4		6.08		3.38
4	4	19.6	16.1	22.8	26.7

[1] Iron was supplied as $FeSO_4 \cdot 7H_2O$.
[2] No molybdenum added, but this concentration of Mo (or V) is estimated to be present in the medium.

Kovats (*29*) has shown conclusively that different preparations of soil humate may vary markedly in the percentage of available molybdenum. The relatively high concentrations of the humates employed furnished optimum iron in all cases unless they were heated with hydrochloric acid (HCl), which apparently largely dissolved out or inactivated the iron of the humates (or their ash) without significantly affecting their molybdenum content. It is now known that in the early work at this laboratory the medium was deficient in available iron, and the strain of *Azotobacter* employed was one that at times has been observed to fix considerable amounts of nitrogen with mere traces of molybdenum in the medium. It is not suprising, therefore, that the preponderant influence of iron in the humates overshadowed

the effect of molybdenum. Bassalik and Neugebauer (*3*) have criticized this early work because they were unable to confirm it fully. They obtained greater effects with soil extracts than with a variety of iron compounds and suggested that the molybdenum content accounted for the difference. The quantity of nitrogen fixed by their cultures was too small to permit definite conclusions, but their suggestion is essentially confirmed by the writers' latest work.

Birch-Hirschfeld (*5*) found that either soil extract or 5 to 250 p. p. m. of molybdenum (0.05 p. p. m. was nearly optimum) gave the same increase in nitrogen fixation by shaken Erlenmeyer flask cultures of *Azotobacter chroococcum*, but that in growth-rate experiments with the Warburg technique only the former was active. The ash of soil extract behaved like molybdenum. The activity of the soil extract on the growth rate was attributed to the organic fraction; and the effect on the ultimate nitrogen fixation, to the molybdenum content. Previous results obtained at this laboratory would indicate, however, that the apparent effect of the organic fraction may be due to the more available iron adsorbed in the organic complex. Ready availability is of course much more important in growth-rate studies than in experiments lasting several days. The failure of molybdenum to stimulate the rate of growth in Birch-Hirschfeld's experiments with the Warburg technique may be attributed to impurity in the medium, which was sufficient for these cultures but not for the heavier Erlenmeyer flask cultures. This is evidenced by the fixation of about 10 mg. of nitrogen per 100 cc. in the absence of added molybdenum or soil extract.

RELATION OF OTHER ELEMENTS TO NITROGEN FIXATION BY AZOTOBACTER

The problem of determining whether additional trace elements are required by *Azotobacter* largely resolves itself into a study of strain variation and purity of the media. These factors, which are likely to vary with the investigator, prevent adequate comparisons and generalizations. Schröder (*34*) has shown the most remarkable insufficiency of molybdenum for several strains of *Azotobacter chroococcum*. When the medium was prepared with distilled water, it was necessary to add molybdenum, zinc, copper, tungsten, and silicon, but when tap water was used the tungsten and silicon could be eliminated without a decrease in growth. Bortels (*11*) has reported some rather inconsistent results, where at times he found that the addition of manganese or silicon or both was necessary to supplement molybdenum or vanadium for maximum nitrogen fixation; at other times manganese and silicon produced no response. Surprisingly enough, he attributes these variations largely to the effects of weather. Flieg (*21*), on the other hand, showed with crude cultures of *Azotobacter* that of 18 trace elements added, only the elimination of molybdenum or iron resulted in significantly decreased growth. The absence of molybdenum had less effect than when iron was omitted, probably because vanadium was still present in the medium.

The earlier work at this laboratory to determine the possible substitution of other elements for molybdenum, in which *Azotobacter vinelandii* V–1 and the Warburg technique were chiefly used, gave no indication of replaceability of molybdenum at a concentration of 10 p. p. m. or less by any of 21 elements tested except vanadium.

Bortels (*8*) has, however, reported some effect of tungsten in certain cases, and Krzemieniewski and Kovats (*30*) have shown that tungsten in relatively greater concentrations will replace molybdenum to a considerable extent in its effect on *A. chroococcum*.

The results of the writers' recent tests of the effects of manganese and tungsten are given in tables 4, 5, 6, and 7.

Table 4 shows no appreciable effect of 0.01 to 1.0 p. p. m. of manganese in the presence or absence of added molybdenum for three strains, except for an approximately twofold effect on *Azotobacter chroococcum* B–8 without molybdenum. With so little growth this is hardly significant.

TABLE 4.—*Effect of manganese on nitrogen fixation by Azotobacter*

[Duration of experiment, 5 days; energy source, 2 percent sucrose]

Species and strain	Nitrogen fixed with indicated amount of manganese (p. p. m.)						
	Without molybdenum				With 1 p. p. m. of molybdenum		
	0	0.01	0.1	1	0	0.01	1
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Azotobacter chroococcum B–8	1.25	1.17		2.42	26.5		28.3
Do	1.39		1.32				
Azotobacter vinelandii V–1	1.21		1.21				
Azotobacter vinelandii B–6	17.10	18.50		14.80	32.6	34.0	30.8

Table 5 compares the effects of equal concentrations of molybdenum, vanadium, and tungsten on relatively old cultures of two strains. At a concentration practically optimum for molybdenum or vanadium (0.5 p. p. m.) tungsten is seen to have but slight effect on total nitrogen or on ratio of nitrogen fixed to sugar consumed for *A. vinelandii* V–1, and no positive effect for *Azotobacter chroococcum* B–8. Vanadium, on the other hand, at the same concentration gives about 60 to 75 percent of the effect of molybdenum on both growth criteria for the two organisms.

TABLE 5.—*Effect of molybdenum, vanadium, and tungsten on nitrogen fixation by Azotobacter*

Experiment No. and species and strain	Age	Element added		Total nitrogen in 100 cc.	Sugar consumed in 100 cc.	N fixed per gram of sugar consumed
		Symbol	Amount			
Experiment 1:	*Days*		*P. p. m.*	*Mg.*	*Gm.*	*Mg.*
Azotobacter vinelandii V–1	19			5.4	1.66	3
	19	Mo	0.5	24.5	2.37	10
	19	V	.5	18.1	2.42	7
	19	W [1]	.5	6.8	1.60	4
Azotobacter chroococcum B–8	33			4.7	.62	8
	33	Mo	.5	35.8	2.09	17
	33	V	.5	27.6	2.11	13
	33	W [1]	.5	2.7	.35	8
Experiment 2:						
Azotobacter chroococcum B–8	19			1.3	.42	3
	19	Mo	1.00	30.8	1.90	16
	19	V	1.00	19.6	1.95	10
	19	Mo	.001	4.4	.71	6
	19	V	.001	3.7	.75	5
	19	[2]		4.0	.64	6

[1] Tungsten was added as tungstic acid neutralized with NaOH.

[2] The energy source in this culture was 2 percent of sucrose 1, which supplied 0.0012 p. p. m. of Mo (or V); the remaining cultures of experiment 2 had 2 percent of sucrose 2; all cultures in experiment 1 had 2.5 percent of sucrose 1.

When much higher concentrations of tungsten are employed, as shown in table 6, the growth of *Azotobacter chroococcum* B–8 and C–4 was highly stimulated in the absence of molybdenum but not in its presence. The total nitrogen of the *A. chroococcum* B–8 cultures was increased about 13 times, or to 15.8 mg. per 100 cc., by 50 p. p. m. of tungsten. This is highly indicative of a spurious tungsten effect caused by traces of molybdenum in the tungstic acid employed.

TABLE 6.— *Effect of high concentrations of tungsten on nitrogen fixation by Azotobacter* [1]

Concentration of tungsten (p. p. m.)	Total nitrogen (mg. per 100 cc.)			
	A. chroococcum B–8		*A. chroococcum* C–4	
	No Mo	Mo (1 p. p. m.)	No Mo	Mo (1 p. p. m.)
0	1.22	25.0	0.86	15.5
1	2.85	26.9	1.60	14.9
10	8.85	26.1		
50	15.80	25.0	3.87	10.0

[1] Cultures analyzed after 6 days; energy source, 2 percent of sucrose 3; tungsten added as tungstic acid neutralized with NaOH.

Table 7 gives the results of an experiment in which four different compounds of tungsten were tested, at several concentrations up to 250 p. p. m. of tungsten, for their effect on nitrogen fixation by the two strains of *Azotobacter chroococcum*. One of these compounds is a sodium tungstate salt that had been commercially purified by the Folin method to remove molybdenum impurities. It is seen that this salt, even in a concentration of 250 p. p. m. of tungsten, caused no significant improvement in the growth of either strain, whereas the other three compounds all gave variable but marked stimulation; the potassium tungstate in concentrations of 10 to 50 p. p. m. of tungsten yielded the maximum growth normally obtained with 1 p. p. m. of molybdenum. Quantitative chemical analyses of these tungsten compounds for molybdenum by the Folin and Trimble method (*22*) gave the following results: Sodium tungstate ($Na_2WO_4.2H_2O$) (Folin), negative (<0.001 percent); $Na_2WO_4.2H_2O$, 0.014 percent; tungstic acid (H_2WO_4), 0.034 percent; and potassium tungstate $K_2WO_4.2H_2O$, 0.125 percent. The indicated impurity in the Folin-purified salt may well have been considerably less than the minimum given. The limit of the test was not accurately determined, but this compound gave an absolutely negative test on a sample several times greater than those of the other compounds. From the analyses of the different compounds just given, the amount of molybdenum furnished by each concentration of tungsten added was estimated and is given in table 7. The amount of nitrogen fixation obtained with the two strains is nearly equal to but slightly higher than that to be expected from the quantities of molybdenum impurity added. Hence, the chief effect from the tungsten compounds is undoubtedly due to their molybdenum impurities. These results also strongly indicate that the marked stimulation caused by sodium tungstate of nitrogen fixation by *A. chroococcum*, reported by Krzemieniewski and Kovats (*30*), was the result of molybdenum impurity.

TABLE 7.—*Effect of tungsten compounds containing various amounts of molybdenum as impurity on nitrogen fixation by Azotobacter*

[Energy source, 2 percent of sucrose 3]

Tungsten added		Mo added as impurity	Mo added as molybdate	Total nitrogen per 100 cc.	
Source	Quantity of W			*A. chroococcum* B-8 [1]	*A. chroococcum* C-4 [2]
	P. p. m.	*P. p. m.*	*P. p. m.*	*Mg.*	*Mg.*
None	0			1.31	1.30
	0		1	32.1	29.7
$Na_2WO_4.2H_2O$ (Folin [3])	1	<0.00002		1.20	1.22
	10	<.0002		1.37	1.27
	50	<.001		1.37	1.36
	50	<.001	1	29.0	26.4
	250	<.005		1.27	1.15
$Na_2WO_4.2H_2O$	1	.0003		2.49	2.05
	10	.003		10.3	4.29
	50	.015		14.1	11.3
	50	.015	1	33.7	
	250	.075		17.2	22.9
H_2WO_4(neutralized)	1	.0005		2.89	2.03
	10	.005		8.81	7.50
	50	.025		19.3	24.8
	50	.025	1	28.7	28.9
	250	.125		25.2	30.5
$K_2WO_4.2H_2O$	1	.0025		8.72	5.34
	10	.025		25.8	26.2
	50	.125		30.7	28.0
	50	.125	1	31.5	
	250	.625		29.1	29.0

[1] Analyzed after 6 days.
[2] Analyzed after 8 days.
[3] This compound of tungsten is a commercially purified salt prepared according to the method of Folin to remove molybdenum impurities. The other compounds are customary c. p. products.

SUMMARY

The influence of molybdenum and vanadium on nitrogen fixation by several strains of *Azotobacter* grown in nonaerated solution cultures was studied.

Eight strains of *Azotobacter chroococcum* and one of *A. vinelandii* showed a tenfold to thirtyfold increase in nitrogen fixation following additions of optimum molybdenum. Two strains of *A. agile* and three of *A. vinelandii* showed only a twofold effect of molybdenum because of a larger fixation in its absence. Maximum nitrogen fixation with optimum molybdenum was, with few exceptions, of the same order of magnitude for all strains tested. Usually this element slightly increased the nitrogen content of the cells.

The quantity of nitrogen fixed per unit of carbohydrate consumed was usually twofold to threefold greater for the *Azotobacter chroococcum* cultures following additions of molybdenum; with the other organisms efficiency was improved to a lesser extent. In the *A. chroococcum* cultures not supplied with molybdenum very little more nitrogen was fixed after an incubation period of 31 days than in 1 week, and usually 50 to 75 percent of the sugar supplied remained unconsumed.

Various samples of sugars supplied about 0.0002 to 0.001 p. p. m. of molybdenum or vanadium impurity when added to media at a concentration of 2 percent. This impurity could be partly removed by filtering through charcoal or by recrystallization from alcohol.

The effective concentration range for molybdenum and vanadium was found to vary from 0.00001 or 0.0001 p. p. m., which gave a detect-

able effect with *Azotobacter chroococcum*, to about 1 p. p. m., which gave maximum growth in 6 days. With younger cultures the range was appreciably narrower, 0.005–0.01 p. p. m. giving maximum growth for 1-day cultures. The concentration range for vanadium was approximately the same as for molybdenum, but the maximum effect was only 50 to 80 percent of that produced by molybdenum.

Experiments dealing with the relation of other elements to molybdenum confirmed the work of previous investigators in showing that both iron and molybdenum must be present in optimum concentrations for maximum growth and fixation. Manganese had no appreciable effect on *Azotobacter* in the presence or absence of molybdenum. Relatively high concentrations of unpurified tungsten compounds were effective as partial substitutes for molybdenum, but this effect could be correlated with the amount of molybdenum present as impurity in these materials.

The essentiality of molybdenum or vanadium for nitrogen fixation by many strains of *Azotobacter* seems to be established by the experiments reported here. Its essentiality for other strains that fix considerable nitrogen in media containing only traces of these elements is, of course, still open to question.

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EFFECTS OF SOME MILD FORMS OF MOSAIC ON POTATO AND A FEW OTHER PLANTS[1]

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INTRODUCTION AND HISTORICAL REVIEW

In nearly all work done on the many different types of mosaic attacking a variety of hosts the more devastating types of mosaic and the hosts of greater commercial importance have commanded most attention.

The reports of the many investigators of the effects of mosaic on the leaves vary with the hosts and types of mosaic. While Smith (*16*)[2] found the yellow parts of mosaic leaves of *Datura stramonium* thicker than healthy leaves, in *Phytolacca decandra* she found the yellowed leaves to be thinner. Schaffnit and Müller (*15*) report mild mosaic spots thinner than normal green parts. Melchers (*14*), working with potato, Iwanowski (*12*), with tobacco, and Dickson (*5*) found the proportion of thickness in light and dark areas approximately 2:3. Although Woods (*22*) makes no numerical comparison of leaf thickness he describes the diseased tissues of the tobacco leaf as consisting of closely packed spongy parenchyma, probably resulting in decreased leaf thickness. According to Cook (*4*), different parts of the same healthy leaf vary in thickness; and comparisons of leaf thickness must therefore be based on many measurements and expressed in very general terms. Certainly where the palisade is much shortened, cuboidal, or undifferentiated and the intercellular spaces are smaller than normal, there can be little doubt as to the decreased thickness of the yellow parts of mosaic leaves (*1*). Probably all deviations from normal in the yellow parts of leaves should not be ascribed to virus. In rugose mosaic, and at times in mild mosaic, the green parts of affected leaves are thicker than the healthy leaves (*16*).

Again, in the matter of size and number of chloroplasts and amount of starch present authors disagree. Cook (*3*) found in sugarcane and tobacco, Westerdijk (*21*) in tomato, Smith (*16*) in *Datura stramonium*, and Hoggan (*11*) in a number of solanaceous plants a reduction in both size and number of chloroplasts in areas affected by mosaic. On the other hand, Dickson (*5*), Woods (*23*), Melchers (*14*), Iwanowski (*12*), and Smith (*16*) noted in various plants a reduction in the number but not in the size of chloroplasts in affected leaves. These infections were probably light, for Dickson (*5*) states that reduction in number precedes reduction in size, and slightly affected plastids show reduction only in chlorophyll. Yet Smith (*17*) mentions reduction only in number of plastids in cells so seriously affected that walls were ruptured and nuclei were disintegrating.

To complete the series of possible variations in chloroplast response to the presence of virus, Doolittle (*6*), working with cucumber, Cook (*4*) with tobacco and tomato, and Clinch (*2*) with potato, found a

[1] Received for publication September 30, 1941.
[2] Italic numbers in parentheses refer to Literature Cited, p. 206.

reduction in size of plastids but not in number. Melchers (*14*) found no difference in number or size of chloroplasts in tomato.

Some investigators believe that the chloroplasts in mosaic leaves gradually degenerate and disintegrate. Köning (*13*) found this to be true in tobacco, Dickson (*5*) and Eckerson (*8*) in tomato, Sorokin (*19*) in tomato, and Hoggan (*11*) in potato in very severe cases. Hoggan (*11*) admits degeneration of plastids only in extreme infections, and Dickson (*5*) mentions degeneration as one of the several effects of mosaic on leaves. Cook (*4*), however, thinks that any degeneration of plastids which others may have observed should be attributed to old age or to causes other than the mosaic virus. Goss and Peltier (*10*) believe that degeneration is not consistent with the recovery or masking so generally observed at 20° C. and above, for which Cook (*4*) says increase in size and number of chloroplasts is responsible.

Iwanowski (*12*) found chloroplasts in mosaic tobacco well filled with starch, as did Clinch (*2*) in the potato. Westerdijk (*21*) found little starch in yellowish plastids of tomato. Dunlap (*7*) found 40 percent as much starch in mosaic tobacco as in healthy tobacco, with small difference between the yellow and green regions of the former. In mosaic leaves starch was more readily converted into simple compounds. Goldstein (*9*) found no starch in plastids of mosaic tobacco. Stone (*20*) determined the lower carbon-fixing and starch-storing ability of mosaic-infected potatoes.

The nucleus is often mentioned in connection with the bodies sometimes associated with the presence of mosaic virus, but the fact that few references have been made to variations in nuclei in mosaic leaves probably indicates that they are not so readily affected as chloroplasts. Cook (*3*) examined nuclei of sugarcane and tobacco and reported no reduction from normal.

METHODS

Study was concentrated for the most part on an important host, the potato (*Solanum tuberosum* L.), but the mosaic infecting it was a very mild and relatively harmless type. Emphasis was placed on the response of the plants to the virus rather than on the identification of the virus. *Solanum* virus 1 was probably present in both the diseased and the so-called healthy potato plants since, according to Smith (*18*), it is almost universally present in the commercial stocks of America though not always apparent. The virus, which in combination with *Solanum* virus 1, produced the symptoms seen in the diseased plants, may very well have been *Solanum* virus 3. Groundcherry (*Physalis* sp.), raspberry, and blackberry (*Rubus* ssp.) infected with mosaic were studied in less detail. No attempt was made to identify the viruses concerned in these plants. The infections are referred to as mild forms in a general sense.

Plants for the investigation were obtained from several sources. The field of a market gardener furnished several mosaic Early Rose potato plants, and another field supplied many mosaic Green Mountain plants. The other mosaic and healthy plants were grown from seed potatoes obtained in the market, some local and uncertified, some from Maine. Healthy plants were obtained from the latter source only. Successive plantings were made beginning in early June. At all times healthy and mosaic plants used for examination or for fixation were

grown out of doors and matched as nearly as possible in age, water supply, light supply, cultivation, and variety.

Fixed and stained material was used for the most part since many of the comparisons to be made were favored by uniform thickness of sections, sharpness of detail brought out only by staining, and the use of pieces too small to be satisfactorily handled as freehand sections.

Fixations were made with Flemming's medium and Allen's B15 solution. A fast green-safranine stain was used in most of the work, but was supplemented by iron haematoxylin in some cases. Fast green-safranine gives good contrast in a healthy cell, chloroplasts appearing brilliant red, cytoplasm pink, nuclei a greenish blue with red nucleoli, and walls a sharp green. The starch does not stain so the grains appear as white spots in the chloroplasts.

Depending on the size of the tissue or organ to be examined, drawn, or measured, 20× or 15× eyepieces and 40× or 60× objectives were used. Drawings were made with the aid of a camera lucida and measurements were taken with a calibrated, movable-scale eyepiece micrometer.

EFFECT OF MOSAIC ON POTATO

LEAF MEASUREMENTS

As stated, the mosaic was a mild type, and it was to be expected that the effects would be correspondingly slight. A series of measurements was made on one set of leaves, taken from the same diseased and the same healthy plants all of one variety (upper section, table 1). Although a decrease in thickness in diseased leaves is progressive, the amount is slight and does not approach the 2:3 ratio which is said to occur in more severe types of mosaic.

TABLE 1.—*Thickness of leaves and palisade layers in mosaic and healthy plants of one variety*

AVERAGE ON FIRST SET OF LEAVES

Plant	Leaf thickness	Palisade	
		Thickness	Total leaf thickness
Mosaic:	*Microns*	*Microns*	*Percent*
Yellow part of leaf	187	62	33
Green part of leaf	190	68	36
Healthy	197	68	35
COMBINED AVERAGE ON FIRST SET AND LEAVES FROM RANDOM PLANTS			
Mosaic:			
Yellow part of leaf	175	58	33
Green part of leaf	181	64	35
Healthy	190	67	35

The measurements on the first set of leaves were averaged with measurements on leaves from 7 other plants taken more or less at random, making a total of over 300 measurements (see lower section, table 1). The paler parts of the mosaic leaves were only 8 percent thinner than similar healthy leaves and only 3 percent thinner than adjoining green parts. In those leaves that were most nearly matched the pale parts were only 5 percent thinner than the healthy leaves and

1.5 percent thinner than the adjoining green parts. Figure 1 shows an exceptionally pronounced example of decrease in leaf and palisade thickness in yellow parts of mosaic potato leaf.

The palisade cells in mosaic leaves appear to have developed to their normal length. The 2 percent difference in proportion of total leaf thickness occupied by the palisade layer in mosaic yellow and normal leaves certainly is not striking.

Groups of mesophyll cells from nine mosaic and healthy leaves were drawn with the aid of a camera lucida and the amount of intercellular space in a given area was determined by a planimeter as follows: Yellow mosaic, 16 percent; green mosaic, 13 percent; healthy, 11

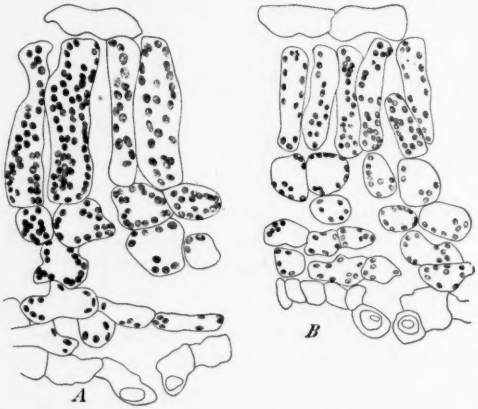

FIGURE 1.—Sections of potato leaves: *A*, healthy; *B*, mosaic. (×550).

percent. In general, the sections of healthy leaf mesophyll showed a smaller area in intercellular spaces than did those of diseased leaves.

SIZE AND NUMBER OF CHLOROPLASTS

Since mosaic is evident as a variation in the amount or shade of green in a leaf it is logical to expect some microscopic differences in the chloroplasts of leaves attacked by mosaic. The pale color of mottled leaves obviously may be the result of one or more conditions: viz, a subnormal amount of chlorophyll in each plastid, smaller plastids, or fewer plastids. No attempt was made in this study to determine the comparative amounts of chlorophyll in the plastids of pale and green leaf parts.

The tendency of normal plastids is to occupy all available space along the wall surface of mature cells so completely as to appear angular from mutual pressure (fig. 2, *A*). Plastids of diseased cells, being not only smaller but also less numerous, are so distributed as to appear rounded (fig. 3).

Samples were taken of a series of mature leaves from a plant in which the yellowing caused by mosaic became progressively more pronounced. Samples from matched leaves of a healthy plant were fixed and stained under the same conditions. Table 2 gives the results of the measurement of chloroplasts from these leaves.

TABLE 2.—*Size of chloroplasts in a series of healthy and mosaic leaves showing simultaneous increase in degree of yellowing and age*

Leaves of increasing age	Average diameter of chloroplast	Comparison of chloroplast size
	Microns	*Percent*
Slight yellowing:		
Yellow part	3.73	9.7 less than healthy.
Green part	3.97	3.9 less than healthy.
Healthy	4.13	
Pronounced yellowing:		
Yellow part	2.89	35 less than healthy.
Green part	3.39	24 less than healthy.
Healthy	4.48	
Severe yellowing:		
Yellow part	1.47	62 less than healthy.
Green part	2.12	45 less than healthy.
Healthy	3.87	6 less than youngest healthy leaf.

The 6 percent decrease in the size of the healthy leaf chloroplast indicates a gradual, slight effect of age on the plastid. However, the size of the plastids in the yellow and green parts of mosaic leaves decreased 61 and 47 percent respectively. No doubt age takes a heavier toll on chloroplasts that are already weakened by mosaic. The chloroplasts of the green parts of the mosaic leaves are more susceptible to aging than those of a healthy leaf. The difference in size of healthy and diseased plastids is noticeable in all the accompanying text figures.

Chloroplasts applied to cell walls were measured in mosaic and healthy potato and tomato leaves. Table 3 shows the length and width of these chloroplasts.

TABLE 3.—*Size of elongated chloroplasts in cells of healthy and mosaic leaves*

Plant	Length of chloroplast	Width of chloroplast	Plant	Length of chloroplast	Width of chloroplast
Potato:	*Microns*	*Microns*	Tomato:	*Microns*	*Microns*
Mosaic	3.75	1.69	Mosaic	5.13	1.98
Healthy	4.06	1.89	Healthy	5.80	2.43

There are several ways of stating the number of plastids in diseased and healthy cells. A set of six plants, mosaic and healthy, was used for one determination of plastid number per cell. Only palisade cells were drawn, together with their plastids. The average number of plastids per cell was as follows: In yellow parts, 41; in green parts, 47; and in healthy leaves, 53; that is, the palisade cells of the yellow parts contained about 13 percent fewer plastids than the adjoining green parts and 23 percent fewer than healthy leaves. Care was taken to match healthy and diseased plants both in age and environment. The differences appearing in size and number of plastids in this set of plants may therefore be considered significant.

Another manner of expressing and comparing plastid numbers is in terms of a unit of area. The areas of a block of cells drawn with the aid of a camera lucida were measured by means of a planimeter. The plastids in these areas were counted and the number of plastids per 100 cm.^{2} of cell area as it appeared on the drawing paper was computed. Eight samples gave the following results:

	Plastids per 100 sq. cm.
Mosaic potato:	
Yellow part of leaf	238
Green part of leaf	225
Healthy potato	192

In the process of fixation the palisade cells of diseased leaf sections seemed to contract somewhat so that the edges of these cells were

FIGURE 2.—Sections of healthy potato leaf showing condition of chloroplasts and starch: *A*, at 9 a. m.; *B*, at 4 p. m.; and *C*, after 20 hours of darkness. ×500.

FIGURE 3.—Sections of yellow part of mosaic potato leaf showing condition of chloroplasts and starch: *A*, At 9 a. m.; *B*, at 4 p. m.; and *C*, after 20 hours of darkness. 8×500.

fluted. This condition might affect the area of the palisade cells, but it would not influence the total number of plastids within each cell. However, it might account for the seemingly high plastid count in unit areas of diseased leaves.

Still a third way of comparing plastid quantity is in terms of area of plastids. If diseased plastids are much smaller than healthy ones,

they will furnish less working capacity even though they are more numerous. Table 4 gives a comparison of plastid size and area of plastids per 100 cm.^{2} in all samples taken, drawn with camera lucida at 1660×magnification.

TABLE 4.—*Comparison of plastid area in a given area of leaf cells of mosaic and healthy potatoes*

Plant	Chloroplasts			
	Diameter	Area	Per 100 cm.^{2} of cells	Area per 100 cm.^{2} of cells
Mosaic:	*Microns*	*Square microns*	*Number*	*Square microns*
Yellow part of leaf	2.8	6.2	225	1,395
Green part of leaf	3.2	8.0	208	1,664
Healthy	3.9	11.9	201	2,392

Though by this criterion the number of plastids in a given area is 12 percent greater in the yellow parts, individually they are only about half as large in area in the yellow parts, with a total area only 58 percent as great as that of the plastids in a corresponding area of healthy leaf tissue. This situation is brought out in figures 2 to 5.

There are two striking differences between mosaic-infected and healthy plastids as seen in a stained section: (1) The smaller diameter of the mosaic plastids, especially in the yellow parts; and (2) the reaction of mosaic plastids to the safranine stain (fig. 5). Healthy plastids stain a brilliant red while those in yellow areas of mosaic leaves and, to a certain extent, those in adjoining green areas do not retain the safranine and so merge into the surrounding haze of cytoplasm.

CARBOHYDRATE FIXATION BY HEALTHY AND MOSAIC-AFFECTED CHLOROPLASTS

If the presence of mosaic virus in the cell does not appreciably change the microscopic appearance of an individual plastid aside from its size, its effect may be apparent in the amount of starch produced by the plastid.

Corresponding leaves on similar mosaic and healthy plants were chosen to furnish material for study of starch production. In the forenoon a green and a yellow piece were cut from a mottled leaf and a piece from the healthy leaf. These were fixed in the same vial, being recognized by shape. In the afternoon a similar set of pieces was taken from the same leaves. The plants were then closely covered to exclude light until the time of the third sampling, the next forenoon. The three sets of leaf pieces were run through the process of fixing and embedding simultaneously and mounted in sets made up either of yellow, green, and healthy pieces at a given time or of yellow, green, or healthy pieces at the three different hours. This procedure prevented variation due to unequal staining and destaining. The fast green safranine stain was used.

At 9 a. m. healthy chloroplasts contained many small starch grains (fig. 2, *A*). At 4 p. m. the starch grains appeared to be much less numerous but were larger; in fact, often a single starch grain so filled the chloroplast that the chloroplast itself appeared as a heavy ring around the starch grain or as a rim on one side of the grain (fig. 2, *B*). After about 20 hours of darkness, during which

the plants were covered, much of the starch had disappeared from the healthy plastids, some appearing entirely devoid of starch (fig. 2, *C*)

At all hours of sampling the small yellow plastids seemed to contain about the same amount of starch (fig. 3). They were never filled to

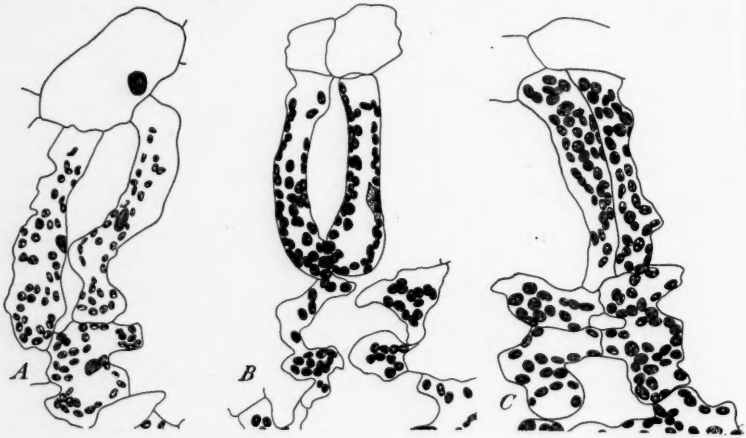

FIGURE 4.—Sections of green part of mosaic potato leaf showing condition of chloroplasts and starch: *A*, At 9 a. m.; *B*, at 4 p. m.; *C* after 20 hours of darkness. × 500.

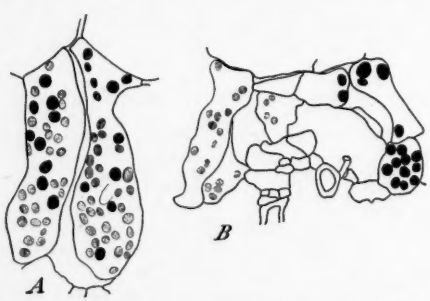

FIGURE 5.—*A*, Cells from green area of mosaic potato leaf showing two sorts of chloroplasts in same cell; *B*, cells from yellow area (left) and adjoining green area (right). × 500.

capacity. Starch grains tended to be small and fairly numerous. The slight differences followed the characteristics of the healthy leaf; i. e., the plastids in the 4 p. m. samples contained the most starch of the three and the plastids in the sample taken after the plant was covered contained the least. Translocation seems not to have gone so far in the mosaic plastids as in the healthy ones. Green parts of mosaic leaves behave much more like yellow parts of the same leaves than like healthy leaves. Starch grains are small and numerous, never filling the plastid, and translocation appears to be even slower than in the yellow parts (fig. 4).

SIZE OF NUCLEUS

Although the nucleus will usually withstand unfavorable conditions as long as any organ of the cell it was thought best to make a series of measurements. Since round nuclei are uncommon, two measurements were taken of each nucleus, length and width, and sizes are so expressed.

The nuclei of cells in the green and yellow parts of mosaic leaves differ little in size, those in the green parts tending to be slightly larger. The nuclei of healthy leaves, however, are considerably larger, being 42 percent wider and 55 percent longer than the nuclei of the yellow parts of mosaic leaves. The upper part of table 5 is made up of about 325 measurements. These were combined with 270 more and the total, approximately 600 measurements, is given in the lower part of table 5.

TABLE 5.—*Average size of nuclei in cells of yellow mosaic, green mosaic, and healthy leaves in a series of 9 matched leaf pieces*

BASED ON ABOUT 325 MEASUREMENTS

Plant	Width of nucleus	Length of nucleus
	Microns	*Microns*
Mosaic:		
Yellow part of leaf	4.5	6.7
Green part of leaf	5.0	6.8
Healthy	6.4	10.4

BASED ON ABOUT 600 MEASUREMENTS

Mosaic:		
Yellow part of leaf	4.8	7.1
Green part of leaf	4.6	7.3
Healthy	6.1	9.6

Table 5 seems to afford sufficient evidence to justify the conclusion that the nuclei of healthy leaves are larger than those of diseased leaves whether green or yellow. Nuclei in healthy and mosaic leaves of corresponding ages were measured at different intervals, but there was no indication of a decrease in size with increasing age. In fact, for the most part the nuclei of older leaves were slightly larger (table 6).

TABLE 6.—*Size of nuclei in leaves of increasing age, 1 indicating youngest leaf*

Condition and age of leaf	Width of nucleus	Length of nucleus
	Microns	*Microns*
Yellow:		
1	4.18	6.64
2	4.45	6.80
3	4.73	6.68
Green:		
1	4.10	6.93
2	5.32	7.03
3	4.52	6.28
Healthy:		
1	6.82	9.91
2	5.92	10.31
3	6.40	11.06

EFFECT OF MOSAIC ON RASPBERRY, BLACKBERRY, AND GROUNDCHERRY

Though the potato has taken the leading role in this series of studies, a few other plants were also used. For epidermal studies material was taken from raspberry, blackberry, and groundcherry (*Physalis*). The mosaics, mild in form, were unidentified.

The mosaic raspberry leaves were strikingly mottled but the surfaces were smooth. Since the lower epidermis of raspberry is hairy and very difficult to detach from the mesophyll only the upper epidermis was examined. The upper epidermis may be more affected than the lower or it may indicate directly the effect of the mosaic on the lower epidermis. However, many groups of about 30 epidermal cells each were drawn with camera lucida, 675× magnification, and the area of the groups determined by means of a planimeter. The results show the area of the upper epidermal cells to be as follows: Mosaic plant, yellow part of leaf, 256 square microns; mosaic plant, green part of leaf, 404 square microns; leaves from healthy plant, 575 square microns.

Both the upper and lower epidermis of blackberry leaves affected by mosaic of two types were measured. Mosaic leaves of the rugose type showed a marked humping of the green areas, the yellow areas remaining smooth. In general the difference in size of the epidermal cells followed the expected course. The lower epidermal cells of the concave surfaces were smaller than the cells on the smooth surfaces, but not significantly so. In the upper epidermis, however, the cells along the contours of the convex surfaces were much larger than those of the adjoining smooth surface. The variation in size in these cells may therefore be considered as due primarily to the contours of the leaf surface, one of the results of the presence of mosaic.

TABLE 7.—*Comparison of epidermal and guard cells in mosaic and healthy blackberry leaves*

Leaf	Area			Stomata per 100 lower epidermal cells	Stomata per 1,000 μ^2 of epidermal cells
	Epidermal cells		Stomata		
	Upper	Lower			
RUGOSE MOSAIC					
Mosaic:	*Square microns*	*Square microns*	*Square microns*	*Number*	*Number*
Yellow, smooth	640	247	235	24	0.97
Dark green, puffed	1,163	224	241	32	1.40
Healthy	660	342	261	33	.96
MOTTLED MOSAIC					
Mosaic:					
Yellow	458	154	210	20	1.30
Green	544	179	190	16	.88

Differences in size of the guard cells in green and yellow areas were negligible (table 7, upper section). No stomata were found on the upper surface of either mosaic or healthy leaves. The number of stomata per 100 epidermal cells was found to be reduced, but in terms of number per unit of area, which, after all, is more important

from the standpoint of their use to the plant, the yellow areas were fully as well equipped as the normal. Again the squeezing together of the cells on the concave lower surface of the green parts of the mosaic leaf accounts, in part at least, for the increased number of stomata in these ares. The reduction in number of stomata in the yellow areas may be directly due to the disease. Decrease in number of stomata might further curtail the production of starch by reducing the amount of carbon dioxide available to diseased plastids.

Leaves of a second type of mosaic blackberry were mottled but smooth throughout. Thus any differences in size of cells may logically be attributed to the mosaic virus. Here only mosaic-affected leaves were used. The lower section of table 7 shows the results of the measurements.

For the study of groundcherry both normal and mosaic leaves were available. The dark green parts of mosaic leaves were only slightly convex, if at all, so any deviations from normal or from yellow areas may be directly related to the disease.

The number of stomata was affected in both green and pale areas, though to a much greater degree in the pale areas (table 8). Here, again, the size of the guard cells remained nearly constant, showing only a four percent decrease in the yellow area. The epidermal cells, on the other hand, showed an average decrease in size of 55 percent in the mosaic leaf as a whole. The decrease in the size of the epidermal cells in the green areas of the mosaic is surprisingly high.

TABLE 8.—*Comparison of epidermal and guard cells in mosaic and healthy groundcherry leaves*

Leaf	Areas in—			Stomata per 100 lower epidermal cells	Stomata per 1,000 μ^2 of epidermal cells
	Epidermal cells		Stomata		
	Upper	Lower			
	Square microns	*Square microns*	*Square microns*	*Number*	*Number*
Mosaic:					
Pale green	623	404	355	13	0.3
Dark green	632	458	357	23	.5
Healthy	1,500	815	369	32	.4

SUMMARY

Width of the palisade layer and thickness of leaf in potatoes are little affected by mild mosaic virus.

In general, intercellular spaces in the diseased leaves of potato are larger than in healthy leaves.

Chloroplasts are reduced in size and number in potato leaves that show mild mosaic mottle.

Chloroplasts in affected areas can be readily distinguished from healthy chloroplasts by their staining reaction.

In chloroplasts of mottled leaves, starch grains are smaller and more numerous than in chloroplasts of healthy leaves. This starch is not readily removed from the plastids during hours of darkness.

The nuclei of mottled potato leaves are considerably smaller than those of comparable healthy leaves.

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Cells in the upper epidermis of yellow parts of mosaic raspberry leaves are appreciably smaller than those of either green parts of the same leaves or of healthy leaves.

Blackberry leaves affected by rugose mosaic show upper epidermal cells in green parts larger than in yellow parts and lower epidermal cells smaller than in yellow parts.

Comparison of size and number of stomata in green and yellow parts of the rugose mosaic blackberry leaf shows no consistent difference.

In blackberry affected with mild mosaic, epidermal cells are smaller in yellow parts than in green, but stomata are larger and more numerous.

In groundcherry, epidermal cells are considerably larger in healthy leaves than in mosaic leaves, but stomata vary little in size or number, at least on an area basis.

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VARIABILITY OF ERODED MATERIAL[1]

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INTRODUCTION

Soils that are subjected to sheet erosion are modified progressively. Productivity is decreased, and measurable changes may occur in the chemical and physical properties of the eroding soil. The changes are due in part to the appearance of subsoil in the zone of tillage. Other soil changes may result from a selective removal of the finer soil fractions by the erosion process. Consideration of the latter point has led to speculation as to the relative severity of erosion losses in relation to quality as well as quantity, and some investigations on the variability of eroded materials have been made.

Middleton, Slater, and Byers (*6*)[2] analyzed mechanically the annual soil losses from various station erosion plots. They found that the texture of the eroded material was finer in some cases than that of the corresponding soil and that it varied with the quantity. In practically every case the material was shown to be appreciably higher than the plot soils in organic content, but in four soils out of eight the mechanical composition of the soil and its eroded material were much the same. These results, therefore, were somewhat at variance with the fact that soil organic matter tends to be concentrated in the finer fractions. The authors of the paper held that their data were insufficient to justify final conclusions.

A more complete study of the mechanical composition of soil losses has been made by Diseker and Yoder (*3*), using the aggregate analysis method of one of the authors. They concluded that—

> In general, soil material is moved layer by layer in the sheet erosion process. The relative loss of colloidal material may be excessive under a condition or combination of conditions which results in small quantities of runoff or in runoff of low velocity or both.

Organic-matter content was not reported.

Rogers (*8*) has reported on chemical variations in eroded materials in a recent study of plant nutrient losses from the Dunmore silt loam. Studies of bacteriological variability have been made by Wilson and Schubert (*10*) and by Norman and Newman (*7*).

The present study was started to clarify certain interrelationships in the variability of eroded material and, more specifically, to determine why organic-matter content fails to follow textural analysis. The point is important, for otherwise textural analysis should govern to a marked degree the chemical and physical properties of the material, insofar as they differ from those of the parent soil.

[1] Received for publication February 25, 1942. Cooperative investigations of the Office of Research, Soil Conservation Service, U. S. Department of Agriculture, and the New York State Agricultural Experiment Station, Geneva, N. Y.

[2] Italic numbers in parentheses refer to Literature Cited, p. 218.

This is evident, in part, from a consideration of the inert character of sands and silts. The difficulties encountered in former attempts to fractionate soil colloids (*2*) complete the evidence by demonstrating indirectly the inadequacy of rainfall or runoff as agents to fractionate the finest soil fractions. Inclusion of organic matter or excessive fines in the eroded material causes it to be different from the soil in other measurable properties, such as rate of carbon dioxide production, or its content of exchangeable bases, but the differences are almost certainly due to the mechanical segregation that has taken place. Except for organic matter, chemical and textural analyses are in effect alternate measurements within the same range of variability. It appears therefore that if the chemical properties of the finer soil separates are known, the variability of erosion losses may be judged solely by the nature of their organic content and textural composition.[3] General chemical examinations have been omitted from the present study.

EXPERIMENTAL METHODS

Five plots were available from which eroded material was collected and analyzed. These were 1/100-acre plots, with metal boundaries, 6 feet wide and 72.6 feet long. The plots are fitted with devices to intercept and collect all soil and water lost by runoff.

The plots are located on Ontario loam, a relatively permeable soil. Plots 1 and 3 were in red clover (*Trifolium pratense*) and alsike clover (*T. hybridum*), respectively, during the course of the experiment. Plot 2 is planted to winter rye (*Secale cereale*) that is turned under as green manure, and has sufficient cultivation to maintain a summer fallow free from vegetation. Plot 4 is planted to soybeans (*Soja max*) annually. Plot 5 is a spaded fallow, with periodic cultivation to destroy vegetal growth.

Sampling of eroded material was accomplished by passing the runoff from each rain through a divider designed[4] for this purpose by Uhland and Woodruff. The use of the divider entailed an undue amount of labor when a large runoff was encountered, but it was entirely satisfactory for the smaller amounts that were usual under local conditions. Preliminary experiments had shown that the divider gave reliable sampling of local soil losses.

Mechanical analyses were made by the pipette method. In the studies on organic matter some mechanical separations were made by wet sieving after dispersion. A combustion furnace was used to determine total carbon, and organic matter was then estimated through the use of the conventional factor, 1.724.

Samples of the plot soils that were analyzed for comparison were obtained at random. Six samples were taken from each plot.

THE DATA

ANALYSIS OF PLOT SOILS AND ERODED MATERIAL

Analyses of the eroded material are based on composite samples that are representative of a year's erosion. The data, together with the texural analyses of the plot soils, expressed as averages of six random samples, are shown in table 1. Organic-matter percentages also are recorded.

[3] The argument is not applicable to losses of applied fertilizers. Granular fertilizers are not inert.
[4] At the Soil and Water Conservation Experiment Station, Bethany, Mo.

The data on 1-year's eroded materials do not give a measure of the degree to which repeated annual data would depart from the textural distributions and organic-matter content that are reported. There is evidence, however, that establishes a reasonable validity for the available data in the use to which they are put. The fact that the samples of eroded material represent from 16 to 35 individual runoffs, and that consistent relationships can be shown to exist between the amounts of runoff and the distribution of the fractions, assures the authors that the major differences exhibited among the eroded materials are not accidental. That these differences are due to sorting, and are not inherent from similar fraction patterns in the plot soils, is established by the fact that the soil-fraction differences between plots do not follow through in the eroded material, even though the soil-fraction differences were found to be significant and highly significant in the cases of sand and silt.

TABLE 1.—*Mechanical analyses of plot soils and of the corresponding material eroded during 1 year*

ANALYSIS OF SOILS

Textural separates (*Mm.*)	Plot cover and No.				
	Red clover 1	Rye-fallow 2	Alsike 3	Soybeans 4	Fallow 5
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
2.0–1.0	2.11	2.39	1.96	1.82	2.67
1.0–0.5	3.53	3.40	3.13	3.36	3.56
0.5–0.25	4.32	4.28	3.93	4.58	4.58
0.25–0.10	14.11	13.46	13.85	14.92	14.18
0.10–0.05	14.90	15.34	14.31	14.96	15.65
Sand 2.0–0.05	38.97	38.87	36.51	39.64	40.64
Silt 0.05–0.002	43.01	43.09	45.12	41.46	40.61
Clay 0.002	18.02	18.04	18.37	18.90	18.75
Organic matter	2.12	1.89	2.16	2.02	1.70
ANALYSIS OF ERODED MATERIAL					
2.0–1.0	2.58	1.89	4.37	2.68	2.28
1.0–0.5	4.75	2.66	6.35	4.64	3.90
0.5–0.25	5.67	3.01	6.66	6.09	3.90
0.25–0.10	17.10	9.55	16.55	15.69	13.34
0.10–0.05	13.95	11.74	11.65	12.69	11.03
Sand 2.0–0.05	44.05	28.85	45.58	41.79	34.45
Silt 0.05–0.002	41.15	51.52	39.85	38.19	44.36
Clay 0.002	14.80	19.63	14.57	20.02	21.19
Organic matter	4.04	2.12	2.92	2.72	1.91

The analyses of the eroded materials show wider divergences than do those of the soils. In comparison with the soil, the organic-matter content of the eroded material is increased in every case, in some cases markedly so. The percentage of sand in eroded material is shown to be as much as 9 percent less or more than the sand content of the corresponding soil. That it should ever be more, on the basis of a year's erosion losses, is somewhat surprising. It is more surprising that the excessive sand content occurs in the soil losses from plots under a cover of red or alsike clover, and that the lowest sand content occurs in the material of the fallow plots. The anomaly is emphasized in table 2, where the percentages of sand in the soil losses are compared to the amounts of soil lost by erosion from the different plots.

In an earlier investigation some data that showed the same trend as that indicated above were discarded by one of the authors on the assumption that sampling had been at fault. In the present case there is no reason to doubt either the efficacy of the eroded-material sampling or the results of the analyses. If the data are accepted, two conclusions must be drawn: (1) The laws governing the transportatior of particles by water cannot be used indiscriminately to account for the composition of eroded material; and (2) the increased organic-matter content of eroded material is not due solely to the presence of increased amounts of silt or clay.

TABLE 2.—*Comparison of sand content of eroded material and corresponding annual erosion losses*

Plot No.	Cover	Sand in soil	Sand in washoff	Soil loss per acre	Rainfall lost as runoff
		Percent	*Percent*	*Pounds*	*Percent*
1	Red clover	38.97	44.05	215	5.96
2	Rye-fallow	38.87	28.85	28,343	11.33
3	Alsike	36.51	45.58	383	5.20
4	Soybeans	39.64	41.79	192	1.35
5	Fallow	40.64	34.45	61,358	18.54

An explanation of textural variability is presented that will be supported later by data on individual soil losses. Under low rainfall and on a permeable soil, conditions are favorable for the total absorption of all precipitation. If, under these conditions, there is any selective movement of fine material, such movement must be downward, mainly through channels and fissures, rather than across the surface of the soil (*1*, *4*). An increase in precipitation to the point where runoff begins does not preclude a continuation of a downward movement of the fines, or a tendency to develop a superficial sandy layer at the soil surface. Consequently, conditions may arise, as has been the case, apparently, on plots 1, 3, and 4, where eroded material actually may be coarser in composition than the eroding soil, as a result of the downward movement of the fines and the transverse movement under subsequent rainfall of the coarser material.

On the plots under investigation where the above-described behavior has been in evidence, erosion losses and the percentages of rainfall lost as runoff have been inconsequential. On the fallow plots where higher percentages of water have been lost in runoff, erosion has followed the pattern ordinarily expected. That is to say, the larger amount of erosion has resulted in material of coarser texture, but in neither case does the composition of the eroded material appear to be as coarse as the material from which it is derived. When the relative quantities of eroded soil from the different plots are considered it appears that under local conditions the general tendency has been to remove the fines selectively.

Complete mechanical analyses of the soil losses resulting from the separate rains have not been made. However, where sufficient amounts of sample were available, a single wet-sieve separation was made at 200 mesh for the separate losses from plot 5. The results of these sieve analyses, together with the data on rainfall and erosion losses are given in table 3.

TABLE 3.—*Analyses of materials eroded from plot 5, and related erosion data*

Dates	Precipitation		Intensity per hour				Runoff		Soil loss			Mechanical separates		Organic matter of separates	
	Amount	Duration	Average	Maximum in—			Depth	Precipitation	Per acre	Per cubic foot of water	Organic matter	Coarser than 200 mesh	Finer than 200 mesh	Coarser than 200 mesh	Finer than 200 mesh
				5 minutes	15 minutes	30 minutes									
	Inches	*Hrs. Min.*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Percent*	*Pounds*	*Pounds*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Jan. 25	0.67	6 : 00	0.11	0.42	0.22	0.20	[1] 1.608	[1] 240.00	1,679.9	0.288	3.41	5.00	95.00	2.36	3.44
Feb. 9	.50	10 : 10	.05	.72	.44	.28	.084	16.80	200.7	.656	3.86	2.50	97.50	5.38	3.65
Feb. 13	.79	11 : 20	.07	.36	.16	.16	.169	21.39	308.0	.503	4.21	1.50	98.50	8.45	4.18
Mar. 23	.32	: 40	.48	2.64	.96	.60	.020	6.25	208.2	2.892	2.60	32.75	67.25	.93	3.13
Mar. 31	.36	: 25	.86	2.04	1.36	----	.164	45.56	4,821.6	8.093	2.68	22.00	78.00	1.67	2.68
Apr. 13	.18	: 15	.72	1.68	.72	----	.108	60.00	2,073.3	5.294	2.39	27.75	72.25	1.65	2.44
Apr. 22	.72	5 : 10	.14	.60	.46	.38	.003	.42	18.4	1.518	1.88	49.25	50.75	1.09	1.96
May 15	.78	17 : 00	.05	.18	.12	.12	.001	.13	20.6	4.790	1.83	57.25	42.75	.69	2.81
June 7	.74	2 : 55	.25	3.60	1.78	1.06	.028	3.78	212.1	2.072	2.06	35.75	64.25	.98	2.60
June 11	.22	: 10	1.32	1.26	----	----	.023	10.45	624.7	7.389	2.29	25.25	74.75	1.19	2.41
June 12	.48	5 : 20	.09	.84	.52	.52	.070	14.58	515.6	2.038	2.23	33.25	66.75	1.11	2.65
June 17	.20	: 45	.27	.84	.36	.22	.002	1.00	17.8	2.917	2.08	42.50	57.50	.74	2.89
June 18	.14	: 08	1.05	1.08	----	----	.044	31.43	793.5	4.948	2.35	25.75	74.25	1.18	2.46
July 14	.42	1 : 30	.28	2.16	.72	.60	.002	.48	64.3	10.153	1.73	47.75	52.25	1.00	2.28
July 23	.15	: 10	.90	1.69	----	----	.005	3.33	79.3	4.659	2.31	21.00	79.00	1.20	2.70
July 23	.34	: 35	.59	2.64	1.04	.66	.028	8.24	354.7	3.488	2.27	31.50	68.50	.95	2.72
July 25	.14	: 30	.28	.84	.36	.28	.002	1.43	30.2	5.368	2.10	36.75	63.75	.65	2.53
July 28	.28	: 30	.56	3.00	1.00	.56	.021	7.50	458.6	5.910	2.36	28.50	71.50	1.05	2.74
Aug. 10	4.46	12 : 00	.37	6.00	3.84	2.96	2.400	53.81	47,864.3	5.494	1.70	32.25	67.75	.66	2.07
Aug. 30	.41	1 : 35	.26	1.96	1.36	.78	.006	1.46	99.5	4.613	2.01	38.75	61.25	.97	2.54
Sept. 12	.33	4 : 10	.08	.84	.48	.28	.003	.91	12.4	1.205	2.85	19.25	80.75	1.40	3.05

[1] Runoff greater than the recorded precipitation was caused by melting snow.

Inspection of these data show that under the small rains characteristic of this location, there is no apparent correlation for the texture of the eroded material with the intensity and duration of rainfall, the soil-water ratio of the runoff, or the amounts of the erosion losses of either soil or water. Elimination of these factors makes it appear that surface conditions, modified by cultivation, and the residues, or the downward movement of colloid that results from prior rain, have been most effective in governing the composition of the eroded material.

Attention should be directed specifically to the data for the storm of August 10. This one rain was outstanding, both with regard to intensity and total precipitation. It accounts for 78 percent of the annual erosion loss. Here the total losses were sufficiently great to discount any minor surface variations. As a result, the texture of the eroded material approximates very closely the texture of the plot soil.[5]

One trend in the data that appears to be fairly definite is that coarser textured material was eroded during summer rains. All the storms that produced eroded material containing more than 35 percent of material coarser than 200 mesh occurred under conditions favorable to permeability between April 20 and September 30, and caused small erosion losses. This behavior is in harmony with the results of the annual erosion on plots 1, 3, and 4 (table 2), where a tendency to produce sandy eroded material was indicated.

Wherever the eroded material contains less than 20 percent of material coarser than 200 mesh, it has been caused by fall or winter rains. One of these (January 25) produced a relatively large amount of erosion. Two other storms that produced a relatively large erosion of fines occurred March 31 and April 13. All three of these storms occurred under like circumstances in that the ground was wet at the time of their occurrence and previous freezing and thawing had tended to destroy the aggregates at the soil surface. Conditions therefore were favorable to the erosion of textural separates. Any tendency of fines to move downward into the soil was at a minimum, and the percentage of rainfall lost by runoff was correspondingly high.

SOURCES OF ORGANIC MATTER IN ERODED MATERIAL

Because the organic matter of soils is known to be associated mainly with the finer fractions, it is not unreasonable to assume that when the organic-matter content of the eroded material is high as compared to that of the eroding soil, the increase in organic matter is due to an increase of fines in the material eroded. This assumption has been strengthened by the fact that high organic matter usually accompanied small runoffs. In data already presented (table 1) evidence is given to show that the assumption is fallacious, that organic-matter content need not reflect textural composition, although obviously textural composition is a factor in determining the amount of organic matter present.

To assume that dispersed soil colloidal material abnormally rich in organic matter has been removed selectively by the erosion process in those cases in which organic-matter content does not result directly from textural composition is contrary to technical experience (*2*). Hide and Metzger (*5*) have shown that relatively high organic matter

[5] See table 4 for comparable soil data.

is associated with the better aggregated soil fractions. If effective differential erosion takes place on an aggregation basis, eroded material should contain less rather than more organic matter than the parent soil. In view of the evidence, the most logical assumption seems to be that the excess organic matter in eroded material, insofar as it is not a result of texture, is due to organic debris removed by erosion.

The data of table 4 support this conclusion to the extent that the annual eroded material coarser than 200 mesh is richer than the corresponding soil fraction in organic-matter content. In these fractions it was evident visually that most, if not all, of the organic matter present consisted of discrete fragments of animal or vegetable origin. The only instance where soil and eroded material contained reasonably similar amounts of such material was on the fallow plot 5, where lack of vegetal cover and highest erosion losses tended to reduce debris effects.

A separation at 200 mesh does not prevent the inclusion of debris material in the finer fraction; consequently it is not surprising to find some rather wide differences between soil and eroded material in the organic-matter content of these fractions. Some of this difference probably is due to varying proportions of very fine sand, silt, and clay, a point that could not be checked in the present investigation. That the differences are due in part to the inclusion of debris is indicated by the fact that the order of organic-matter content in the eroded material is the same in both the fine and the coarse fractions. Moreover, on the fallow plots, 2 and 5, where greatest erosion occurred, the organic-matter content of the fine fraction of the eroded materials approaches the limits set by the analyses of the fine soil fractions.

TABLE 4.—*Organic-matter content of coarse and fine fractions of the plot soils and of the corresponding material eroded during the year*

Plot No.	Organic matter	Mechanical separates		Organic matter of separates	
		Coarser than 200 mesh	Finer than 200 mesh	Coarser than 200 mesh	Finer than 200 mesh
ANALYSIS OF SOILS					
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
1	2.12	30.5	69.5	0.98	2.44
2	1.89	30.0	70.0	.43	2.40
3	2.16	28.0	72.0	.97	2.77
4	2.02	30.5	69.5	.61	2.68
5	1.70	30.1	69.9	.56	1.95
ANALYSIS OF ERODED MATERIAL					
1	4.04	41.5	58.5	2.74	4.71
2	2.12	22.0	78.0	1.26	2.31
3	2.92	42.0	58.0	1.89	3.47
4	2.72	33.0	67.0	1.45	3.22
5	1.91	30.1	69.9	.79	2.24

Enough samples were available from plots 2 and 5 to make separate analyses of the 200-mesh material from the rain of August 10. The erosion occasioned by this rain was sufficient to mask debris effects. The material from plot 2 ran 2.27 percent and the material from plot 5 ran 2.07 percent in organic-matter content.

In table 3, data from the type of analysis just discussed have been reported for the sieve fractions from the plot 5 individual soil losses. Here, where effects of cover are absent, there is an evident relationship between organic-matter content and the proportion of the textural separates present in the eroded material. But there is evidence of debris effects also in the high organic-matter content of some of the coarser fractions. The source of this may be wind-borne material. This plot had been kept completely fallow for 3 years prior to the time of this investigation.

It has been emphasized that a constancy of colloid composition may be assumed in dealing with the eroded-material fractions. To test the point and show that a high organic content in the fines of this material is in no way associated with the segregation in the eroded material of inorganic colloidal material especially rich in organic matter, some colloidal material was extracted both from the soil and from a series of soil losses. Duplicate extraction and analyses of soil colloid gave organic-matter content of 3.17 and 3.32 percent; colloid from separate soil losses ran 3.14, 3.29, 3.34, and 2.96 percent.

DISCUSSION

The results of the present study, together with those of former investigations, furnish a basis for evaluating the variability of eroded materials. Evidence that is available indicates that under severe erosion the eroded materials tend to approximate the composition of the eroding soil, that it is in effect "removed layer by layer." With more moderate runoffs there is a selective removal of the finer fractions. Small local depositions of sand on the soil surface may be swept off by later rains, but if frequent cultivation constantly presents a fresh surface to the sorting action of running water, a continued removal of fines may be expected.

On relatively permeable soils, where infiltration represents a high proportion of the total precipitation, the downward movement of fines apparently results in a superficially sandy surface layer. The removal of this material by runoffs of slightly greater intensity results in small erosion losses of relatively coarse texture.

The widest range of composition between soil and eroded material is to be expected from loams or sandy soils. The removal of "finer fractions" by erosion from a soil composed entirely of silt and clay seems highly improbable. Moreover, the most effective sorting by comparable runoffs may be expected to take place on weakly aggregated or single-grained soils, since the removal of textural separates implies a break-down of the texturally heterogeneous soil aggregates.

That soils generally are not markedly affected texturally by the variability of eroded materials is evident in part from the fact that the heaviest erosion losses show the least variability. A simple example will illustrate the effect of smaller losses. An erosion loss of 10 tons per acre that contains 10 percent less sand than the original soil results in an accumulation of sand of but 1 ton per acre, or enough to increase the sand content of the surface soil by about one-tenth of 1 percent.

The silt and clay content obviously drop by the same amount, but changes of this order are negligible, except on excessively sandy soils or for their cumulative effect. The data of Scarseth and Chandler (*9*)

illustrate the point. These investigators found that under the conditions of their experiment, a selective erosion of fines took place that amounted to only 3 percent of the whole soil, but nevertheless was 33.3 percent of the clay fraction. To infer, however, that continued removal of excess fines by erosion must inevitably cause a surface soil to become lighter in texture is fallacious. That can happen only if the subsoil that replaces erosion losses is lighter in texture than the eroded material.

Because of this circumstance the assumption should not be made that erosion losses always are relatively more damaging if they contain high percentages of fine materials. The assumption is based on the fact that the available plant nutrients of the soil are confined mainly to the finer fractions. It overlooks entirely the equally well-known fact that soil structure and tilth, which are dependent in part on texture, also are important in maintaining productivity. Podsolic soils generally tend to become heavier as the result of erosion. On soils of this type, particularly on those that have a low organic content, experience may show that a selective removal of fines is less damaging than the removal of the same amount of sandy material.

The point determined in the present investigation, that small erosion losses may be sandy in character, may not be of more than theoretical interest because of the small amounts of erosion that are involved. It seems to bear, however, on the data presented by Hester and Shelton (*4*), which show a downward movement of silt and clay from the surface horizon of a soil that amounted to 79 tons per acre, as the result of long-time cropping and fertilizer practices. This result contrasts so markedly with the results obtained by Scarseth and Chandler that it seems necessary to postulate essentially different types of erosion for the two experiments.

The losses of organic matter caused by erosion are variable both in amount and character. They tend to be high in proportion to the total amount of soil and the proportion of fines that are lost. It is to be presumed, however, that the loss of well-decomposed and relatively stable humus intimately associated with the organic-inorganic soil complex, closely follows the textural composition of the eroded material. Losses of organic matter in excess of this amount may vary in character from bacterial gels and the mycelia of fungi to coarse and fibrous residues of vegetation. Small runoffs contain higher proportionate amounts of such materials.

These assorted organic materials may be characterized as the more active fraction of the eroded organic matter. The variable results of such losses on the remaining soil may be judged by the effects of applications of similar amounts of similar materials, litters, manures, or composts.

It is well known that organic materials applied to the soil tend to disappear in a relatively short time as a result of bacterial decomposition and oxidation. The organic debris that is carried away by erosion undoubtedly is destined for the same fate, and its presence therefore tends to exaggerate the permanently characteristic differences that distinguish a soil from its eroded material. The removal of active organic matter of high fertility value may have an immediately deleterious effect on the remaining soil of somewhat temporary character.

Losses of relatively stable humus are cumulative in effect, and therefore are of greater importance. Soil humus is not replaced easily. Its capacity to release food and energy for plant growth and bacterial activity is small as compared with that of green manures or freshly applied composts; nevertheless it maintains an ability to serve as a storehouse of plant food by reason of its amphoteric character and high base-exchange capacity, and so regulates the supply of nutrients available for plant growth. Equally important, it affects the physical character and tilth of the soil, and tends to buffer and extend the zone of conditions favorable to maximum plant growth. The variability of eroded material that supports an excessive loss of fines becomes relatively more damaging in proportion to the rate at which it lowers the humus content of the remaining soil.

SUMMARY

A series of soil losses and the corresponding plot soils have been analyzed texturally and for their organic-matter content.

Since rainfall was light, soil and surface conditions, rather than intensity and duration of rainfall, appeared to govern the textural composition of the eroded material. Under conditions favorable to infiltration and downward movement of fines, eroded material has been produced that is coarser in texture than the corresponding soil; under conditions of greater impermeability, eroded material has been produced that is finer in texture than the corresponding soil.

These differences tend to disappear as erosion increases, and the composition of the eroded material approaches the composition of the soil.

Argument has been advanced to show that a disproportionate removal of fines need not be more serious than the removal of the same amount of soil in its total.

Irrespective of texture, eroded material has been shown to be somewhat higher in organic-matter content than the eroding soil, especially where small runoffs are incurred. This anomaly has been shown to be the result of organic debris that is removed preferentially by the eroding process.

If the effect of organic debris is discounted, colloidal material removed by erosion has essentially the same organic-matter content as the colloidal material of the eroding soil.

The textural separates of a soil are essentially identical with comparable separates from its eroded material and, except for deviations due to organic debris or temporary physical conditions, all properties in which eroded material may differ from the soil must be the direct result of the relative proportions of separates that are included in the material.

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